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Gastric digestion of soybean flour when used as a substitute for cows' milk in feeding dairy calves

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GASTRIC DIGESTION OF SOYBEAN FLOUR WHEN USED AS A SUBSTITUTE FOR COWS' MILK IN FEEDING DAIRY CALVES

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One of the sources of continual worry for the dairy farmer and one which has to do with the very life of the industry, is the problem of the economical rearing of calves. This is a serious problem largely because a portion of the marketable product of the dairy enterprise - milk - is required for feeding the young calf. A vast amount of work has been done to produce calf feeds which may displace milk in the calf ration. Many stations have been outstanding in their interest in the subject of calf meals and gruels, while practically every station has at some time considered some phase of the broad problem of milk substitutes. Interest has been shown in these attempts because the production of a practical and efficient substitute for milk for calf feeding would effect an immense saving to the dairy farmer. Such would be of especial value to the producer of whole milk.

The problem has been attacked in most cases along one or the other of two lines, (1) the use of factory by-products of the processing of milk itself in butter and cheese making, the feed products thus produced being dried skim- and butter-milk, and semi-solid skim- and butter-milk, or (2) the substitution of cereals, hay, starch and sugar, and packing house by-products such as beef extract, beef blood, meat, and bone meal, as a part or all of the calf ration.

Quite satisfactory results have been obtained with dried- and semi-dried milk products, (83, 25, 30, 23, 81, 26, 10) since upon reconstitution with water they assume many of the properties of the original fresh milk. Moreover the dried milks have the rather important characteristic of not spoiling easily and thus being adapted to reasonable transportation and storage. They have the disadvantage of being somewhat expensive so that their use has not become so general as it would, could they compete more easily with fresh products on the point of cost.

The substitution of products of other than milk origin continues to receive the attention of a great number of investigators. One of the most serious phases of this problem is finding efficient substitutes for milk which contain a low enough content of indigestible material, which the digestive systems of very young calves find difficult to handle. A compilation of several calf meals disclosed fiber contents of from 2.3 up to 6.8 per cent.

Another fact which must be considered in preparing a substitute for milk is that the amino acid content of casein, one of the proteins of milk, is near ideal in its balance for the development of calves, and that the same array of amino acids in their particular percentages is not found elsewhere in a single protein. Again, the action of rennin in curdling milk protein into a solid mass almost immediately after the milk reaches the stomach, thus literally transforming a liquid diet into a solid one and thereby possibly preventing its too rapid passage into the intestines, is apparently not extended to any other protein.

Since there is no single protein with the exact constitution of the milk protein, it has been necessary in obtaining a substitute, to bring together various grains, cereal by-products and packing house by-products, some of which are difficult to obtain. Thus while some calf meals may produce satisfactory results, the farmer may yet find it a difficult task to assemble all of the necessary ingredients for the mixture he has decided to use.

According to Lindsey and Archibald (57) there are four important factors to be considered in formulating a calf meal: (1) variety and completeness of proteins, (2) sufficient carbohydrates to balance the ration satisfactorily, (3) plenty of vitamins, and (4) sufficient mineral matter. This requirement is in line with the earlier recommendations of McCollum (59) who gave as the essential qualities of an adequate diet: (1) adequate proteins, (2) a source of energy in the form of proteins, carbohydrates or fats, (3) a suitable supply of certain inorganic salts and (4) "two as yet unidentified substances or groups of substances", the latter group referring of course to vitamins.

It is usually hoped that the first factor will be cared for by the use of a high protein concentrate such as linseed meal, beef blood or powdered skimmilk. From a total of forty observed calf meal formulae, linseed or flaxseed products were mentioned in thirty-five mixtures, beef blood or blood flour occurred in nine cases and dried skimmilk was included in seven formulae. The great popularity of linseed and linseed products in calf meals is not due entirely to their protein content nor yet to their ease or completeness of digestion. The tendency for gruels to pass rapidly through the digestive tract is supposedly prevented to a great extent by the sticky or gummy characteristic of linseed products, especially after cooking. Powdered skimmilk also retains, to an extent, the coagulation characteristic of fresh milk, due to the casein which is in it.

The carbohydrate content is easily cared for by the addition of cereal grains to the mixture. Corn, oats, wheat, etc., furnish ample carbohydrate material. A few calf meals have been assembled with a certain content of starch or sugar. Shaw, Woodward and Norton (85) demonstrated the ability of the young calf to digest starch.

The need for fat is supplied by the oil content of grains and in some cases by the addition of corn oil and other oils.

The mineral requirement is met by use of bone meal, calcium and phosphorous compounds which are added to the mixture, while the vitamin requirements are cared for by use of cod-liver oil. Additional vitamins are also obtained through feeding some form of roughage which is high in vitamin content, either added to the meal mixture or fed separately.

Although comparatively fair growth is obtained through feeding certain calf meals, one wonders why milk-fed calves are apparently healthier and grow better than those fed on meals. Several factors may influence the results obtained with a milk substitute. As has been mentioned before, all calf meals contain fiber, which is difficult for the young calf's stomach and intestinal tract to handle. The ration may also lack palatability so that the calf does not consume the amount of nutrients that it would consume in milk feeding. If provision is not made for holding the food in the stomach for sufficient time for peptic digestion to get well under way, scouring may result, which, according to Mann (60) decreases the digestion coefficient of all nutrients except nitrogen-free extract. Finally the very complexity of many mixtures makes them of limited value as milk substitutes. There is a tendency on the part of the feeder to leave out some ingredient which is difficult to obtain but which may be vital to the ration. If such rations are factory-mixed, the cost is often prohibitive.

It would appear then that the mixture which comes nearest avoiding all of the above criticisms, should be most useful as a calf meal.

The Soybean: Its Composition and Nutrient Content

A glance at the composition of soybeans shows a rather close resemblance between the constitution of this material and cow's milk, which would suggest the possibility of their substitution for milk as a feed for young dairy calves. The following table (table I), the data for which were taken from Henry and Morrison (39), shows the analyses of the two products:

TABLE I

PERCENTAGE COMPOSITION OF SOYBEANS AND COW'S MILK

	Dry matter	Ash	Crude protein	Carbohydrates Fiber	Carbohydrates N.F.E.	Fat
	%	%	%	%	%	%
Soybeans	90.1	5.3	36.5	4.3	26.5	17.5
Skimmilk	9.9	0.7	3.8		5.2	0.2
Whole milk	12.8	0.7	3.5		4.9	3.7

The resemblance becomes more striking if the milk is reduced to a dry matter basis. In table II a comparison is made of soybeans with whole milk, dry basis, the figures being taken from Henry and Morrison. In this table it is shown that in total protein, carbohydrate, fat and ash contents, soybeans and whole milk are quite similar. Milk of course contains no fiber. A comparison of the nutrient content of the two products (table III) shows that in this respect the two materials are rather similar, the protein being somewhat higher, and the carbohydrates and fat, somewhat lower in the soybeans than in cow's milk.

TABLE II

PERCENTAGE COMPOSITION OF SOYBEANS AND WHOLE COW'S MILK (DRY BASIS)

	Dry matter	Ash	Crude protein	Carbohydrates Fiber	Carbohydrates N.F.E.	Fat
	%	%	%	%	%	%
Soybeans	90.1	5.3	36.50	4.3	26.50	17.5
Whole cow's milk	100.0	5.5	27.34		38.28	28.9

TABLE III

DIGESTIBLE NUTRIENTS OF SOYBEANS AND WHOLE COW'S MILK (DRY MATTER BASIS)

	Dry matter	Digestible protein	Carbohydrates	Fat
	%	%	%	%
Soybeans	90.1	33.2	24.7	16.1
Cow's milk	100.0	24.3	35.3	26.5

The Protein of the Soybean (Glycinin)

Inasmuch as the protein content of the ration of the young animal is of paramount importance, a consideration of the quality of the soybean protein, or rather of the constitution of glycinin is well in order. The real value of a protein is determined by its amino acid content, and since casein appears to be an entirely satisfactory protein for the young animal, its make-up of amino acids may well be studied in seeking a substitute. In table IV are listed the amino acid contents of cow's milk (casein) and soybeans (glycinin), as given by Mitchell and Hamilton (62). Only the amino acids, arginine, histidine,

TABLE IV

AMINO ACID CONTENTS OF SOYBEANS AND COW'S MILK (CASEIN)

	Arginine	Histidine	Lysine	Tyrosine	Tryptophane	Cystine
	%	%	%	%	%	%
Soybeans: glycinin	15.3	2.4	10.3		1.3	0.8
Cow's milk: casein	7.4	6.2	10.3	2.7	1.9	0.2

lysine, tyrosine, tryptophane and cystine are considered in these data. Holmes (43), Rittinger and Dembo (77) and Hill and Stuart (40) all give a tyrosine content for soybeans, of 1.86-1.90 per cent. Holmes also gives a valine content of 0.68, a glycine content of 0.97, an ammonia content of 2.56, a leucine content of 8.45, a proline content of 3.78, a phenyalanine content of 3.86 and an aspartic acid content of 3.89 per cent. Stuart and Hill give similar figures except that they give a figure of 19.5 for glutamic acid, and no content of proline.

Morris (64) gives a value of 1.46 per cent for cystine for soybeans and 1.25 for fresh skimmilk. For skimmilk powder he gives a cystine value of 2.04 per cent.

Mitchell and Villegas (63) found soybeans to have a biological value of 64 as compared to a value of 58 for corn, 67 for rice bran, 59 for coconut meal, 66 for cottonseed meal, and 62 for alfalfa hay.

Holmes (43) reported, upon the basis of experiments with laboratory animals that "while it was found that under some conditions, tyrosine, cystine, arginine, histidine, glutamine and aspartic acid may act as limiting factors in the diet, it seems to be very generally recognized by these students of nutrition that the two amino acids, lysine and tryptophane are of especial importance in the dietary, lysine being essential for 'growth' and tryptophane essential for 'maintenance". He stated further that "investigations studying the biologic value of these proteins by experiments with laboratory animals have shown that soybean and peanut proteins where employed as the sole source of protein in an otherwise adequate diet, support in a satisfactory manner the normal body processes of growth, maintenance and reproduction".

Daniels and Nichols (19) reported that "the value of the protein of the soybean has been demonstrated in all our experiments. That animals fed rations containing 15.6 and 18.7 per cent of protein obtained solely from the soybean have grown normally and in the latter case have produced successive litters of young, which in turn have reproduced, is sufficient evidence that the protein of the soybean fulfills all physiologic requirements. The protein of the soybean appears to be quite as valuable as the casein of milk".

Soybean Flour

Because of the ever-increasing uses of the oil of the soybean for purposes other than livestock feeding, economy has demanded that the beans be fed in an extracted form, i.e. without the oil. Thus the by-products of the soybean oil industry are becoming the more common soybean feeds.

The residue from the oil extraction is usually left in cakes, which in turn are ground to various degrees of fineness ranging from lumps the size of a pea down through the coarse meals, known as soybean oil meal, to a fine bolted powder which is called "soybean flour". While the coarser meals and "cake" are the more common stock feeds, the flour has been used considerably for human consumption.

Naturally the extrgction of the oil from the soybean material leaves a product of considerably different composition, when the percentages of its various constituents are considered. Table V shows a comparison of the composition of soybean flour with that of dried skimmilk. This table shows a rather close similarity between soybean flour and dried skimmilk. If the soybean flour is considered as mixed with water in the ratio of flour, when diluted with water and used as a substitute for milk in the diet of a young dairy calf has a similar gastric digestion to that of milk.

REVIEW OF LITERATURE

The great mass of data which now exist on the subject of milk substitutes for calves, has accumulated during the thirty-five years which have elapsed since Crawford (18) in Australia, reported in 1900, his investigations on the use of oil cake, oatmeal and hay tea as a substitute for milk.

Hayward (38) 1902, at the Pennsylvania station was among the first in this country to report work on calf meals or milk substitutes. He concluded that "there is little difficulty in raising prime dairy calves without milk after they are two weeks old". He reported the use of a ration composed of wheat flour, coconut meal, dried skimmilk, linseed meal and dried blood, fed as a gruel.

The following year Lindsey (54) reported his early work at Massachusetts, dealing with calf meals as substitutes for milk. He reported the results of a single trial with a calf fed on a calf meal. He observed that "it will probably prove necessary to feed one-third skim- or whole milk during this period" - referring to the first three months of the calf's life.

Haecker (34) 1905 at Nebraska reported that "linseed meal is not only an excellent food for replacing butterfat in skimmilk for calf feeding, but also an economical food in comparison with others".

Savage and Tailby (83) 1909 at Cornell reported work upon the use of substitutes for skimmilk in calf feeding. They stated in part that "the heredity of the animal is of more consequence than any moderate influence in the care or feeding for a short time at the beginning of the development of the individual". They said further that "if the calf is not stunted permanently by continuing poor feed for too long a time, the lack of thrift which might result from using a substitute for milk from the time the calf is ten days or two weeks old until he is on hay and grain entirely, may be overcome in the later development of the animal if the dry food given from the time he is five months of age is proper and sufficient in amount".

Foulkes, Andrews and Garnett (29) 1914, in England, fed calves on milk substitutes including linseed, linseed meal, flour, bean meal and oatmeal, which resulted in body gains of from 0.21 to 1.45 pounds daily.

Lindsey (56) 1915 developed a number of calf meals which he used in thirteen different trials. While feeding these various calf meals he obtained gains of from 0.87 to 1.70 pounds per day in his calves.

Hunziker (48) 1917, reported increased body weights of 0.73 to a pound a day with calf meals supplemented by small amounts of milk.

Gillette, McCandlish and Stange (32) 1918 recommended that "where milk substitutes must be used it is probably best to wait until the calves are six or eight weeks old and then substitute them gradually".

The same year, Shaw, Woodward and Norton (85) reported trials in which starch digestion in the young calf was determined. They concluded that "these results indicate that the milk ration of a calf but a few days old may be supplemented with starchy food and that the starchy material may be rapidly increased as the calf grows older".

Honcamp and Dettweiler (44) 1919 fed skimmilk with ground oats and flaxseed to calves which made 90 per cent of the gains of calves fed on whole milk.

Bruce (12) 1920, in Australia used various grain mixtures of linseed meal and bean meal as gruels in feeding calves which produced average daily gains of 1.36 to 2.3 pounds.

Spitzer and Carr (91) 1920, reported work involving the development of a calf meal consisting of beef blood, corn and linseed oil meal from which they secured gains in calves, of 1.18 pounds per day.

Ellington (25) 1922, reported average daily gains of from 1.12 to 1.45 pounds on various calf meals containing corn, alfalfa and linseed oil meal. It is interesting to note his statement that "there is no danger of scouring after the change is complete", referring to the change from milk to calf meal.

Ragsdale and Turner (75) 1923, reported work to determine the minimum milk requirements for calf raising. They concluded that "growth at a rate approximately 70 per cent of normal for calves under six months of age can be secured by weaning thrifty dairy calves when 60 to 70 days old and substituting therefor a good quality of alfalfa or soybean hay and a suitable grain mixture". They said further that "following the change to grain and hay, poor growth is made until the calves become accustomed to the dry feed. After the first two months on such a ration there is a tendency for large gains to be made. However, the gains are not large enough to enable the calves to return to normal weight and height before they reach six months of age". They concluded that "with few exceptions the Holstein calves reach normal weight by the eighth or ninth month while the Jerseys reach approximately normal weight by the age of one year".

Eckles and Gullickson (23) 1924, reported that "a careful study of the entire question led to the conclusion that the practical solution of the problem of raising the calf when whole milk is sold is not to attempt to find a milk substitute but to use the least possible amount of milk".

Maynard, Norris and Krauss (61) 1925, reported the use of a calf meal containing corn meal, flour, oat groats, linseed oil meal, barley, blood flour and minerals, from which they obtained average daily gains of 1.44 to 1.67 pounds to the age of six months.

Lindsey and Archibald (57) gave the factors which they considered of importance in formulating calf meals as follows:

1. Variety and completeness of proteins.
2. Sufficient carbohydrates to balance the ration satisfactorily.
3. Plenty of vitamins.
4. Sufficient mineral matter.

They also recommended the substitution of skimmilk for whole milk at the age of 10 days and the starting of calf meals at two to three weeks. Theirs is one of the most extreme of all recommendations since most workers have suggested that from a month to eight weeks is probably the proper age to begin the substitution of calf meals.

Archibald (5) 1928, upon the basis of feeding trials, concluded that neither cooking nor very fine grinding appeared to improve the digestibility of calf meals.

Bender and Perry (9) 1929, recommended the beginning of calf meals at ten days of age and that at thirty days calves should be on calf meal and hay entirely. Their recommendation is in line with that of Lindsey and Archibald, mentioned above.

The Michigan station (30) 1930, reporting work with calf meals concluded that calves can be successfully raised on not more than 180 to 280 pounds of whole milk, and that by the time the above amount of whole milk had been used, skimmilk and calf meals could be substituted entirely for whole milk.

Blackshaw (11) 1930, reported that a "calf should not be completely off milk until seven or eight weeks old". This is a much more conservative recommendation than those of Archibald and Lindsey, and Bender and Perry.

Again in 1930, Lindsey and Archibald (56) reported that "while it is possible to grow Holstein calves by the dry method of feeding, it is not to be commended". This recommendation, apparently supported by continued experiments, indicates a rather radical change of opinion regarding the virtues of the dry feeding method. "Most of the calves", they continued, "appeared thin, 'pot-bellied' and not in as satisfactory condition as those receiving a portion of natural skimmilk or dilute skimmilk powder until four months old. It is believed very young calves should be given a vigorous start by feeding some form of milk as a part of the ration until they are at least four months of age. They should not be converted into 'cud chewers' too early".

In line with the conservative recommendations following the more recent research on the subject of calf meals, Jones, Brandt and Wilson (51) 1931, concluded that "calves can be successfully weaned from milk at from 30 to 50 days of age, depending upon the size and vigor of the calf. A small amount of milk fed over a longer period is preferable to the same amount fed over a shorter period". They secured an average daily gain on calf meals of 1.24 pounds. They concluded further that "the use of a dry calf meal in raising calves in comparison to whole milk, skimmilk, grain and hay, lowers the feed cost of raising a calf to 180 days of age from ten to fifteen dollars. In addition to the saving in feed cost, the system requires less labor".

Cannon (13) 1931, found that while calves fed whole milk alone tended to develop rickets and anemia, the addition of grain and alfalfa dispelled any symptoms of these disorders. His findings indicate that while whole milk is an ideal food for very young calves, it ceases to be ideal for calves after they have reached the age of five or six months, for then it should be supplemented.

Berry (10) 1931, reported that "in following any minimum milk plan in raising calves, they may be weaned from milk at 45 to 60 days of age, depending on the size and vigor of the calf. Small calves should not be taken off milk until they are 60 days of age". He stated further that "the continued feeding of whole milk is not economical. Other methods produce as satisfactory calves".

Savage and Harrison (82) 1933, reported that "Holstein calves made very creditable growth when fed 350 pounds whole milk during the first seven weeks, a dry calf-starter from three weeks to sixteen weeks of age, a 12-per cent protein concentrate mixture from the seventeenth to the twenty-sixth week and mixed hay throughout the entire six months period". They were optimistic regarding milk substitutes, however, for they stated further that "on the whole, trials at this station with calf starter mixtures have indicated that feeding with these mixtures can be considered as one of the recognized methods in raising dairy calves".

From South Carolina, Elting and LaMaster (26) 1934, reported that "if calves are given a good start, they may be weaned from milk at 50 to 60 days of age. The growth rate may lag at first but usually it is regained by the age of six months". They concluded, however, that "limited-milk fed calves develop a skeletal structure of somewhat lower density and breaking strength than calves receiving skimmilk to the age of six months".

Krauss, Monroe and Haydon (53) 1935 compared calves fed calf meals with calves fed skimmilk. They reported that "all groups made satisfactory gains from 1.42 to 1.96 pounds daily". They concluded that "because during the second, third and fourth months, dry-fed calves are apt to look rather pot-bellied and shaggy, a breeder who is anxious to have all his stock looking at their best all the time may find this system undesirable. By the time the calves are six months old, their physical appearance is good, although their finish is not quite equal to that of calves raised on liquid skimmilk. At the end of a year, it is impossible to distinguish animals raised on a dry-feed system from heifers of the same age raised on liquid skimmilk".

Recent work by Savage and Crawford (81) 1935 indicates that "dry skimmilk calf starter is a good feed for calves. When fed according to feeding schedules followed in these trials, calves may be expected to make satisfactory gains. All Holstein calves in these trials, which were fed calf starter mixtures containing dry skimmilk, gained, on the average 1.56 pounds daily". They stated further that "no back-weakness was observed in calves which received 0.5 per cent reinforced cod-liver oil in their calf starter mixture". This starter contained 20 per cent of dry skimmilk.

According to Eckles (22) the average weight at birth of Holstein calves is 90 pounds and the average weight at six months of age is 349 pounds, the average gain for 180 days being 259 pounds or approximately 1.44 pounds per day. Upon the basis of these figures many of the experiments reported have produced calves making "normal" growth by use of calf meals. On the other hand it should be remembered that many of the results reported could not be considered satisfactory so that the present array of calf meals for feeding either dry or as gruels must be considered as far from ideal substitutes for milk - the natural food of the young calf.

Soybean Products as Feed Material

The use of soybeans and soybean products for stock feed is not new. Humphrey (47) 1904 reported trials at Wisconsin involving a comparison of soybeans with middlings as a supplement for fattening pigs.

Since 1900 the use of soybeans and soybean products has spread rapidly so that they are now commonly used, high protein feeds. The greatest impetus to using soybeans, both from the standpoint of livestock feed and human food, was given during the World War.

Williams and Park (102) 1917, reported that "soybeans are equal in feeding value to cottonseed meal and linseed oil meal". They gave the following data concerning the digestibility of soybean meal as compared to other high protein feeds (table VII):

TABLE VII

DIGESTIBILITY OF SOYBEAN MEAL AS COMPARED TO OTHER HIGH PROTEIN FEEDS

	Total digestible nutrients	Assimilability	Digestible albumin	Starch equivalent
	%	%	%	%
Cottonseed oil meal	76	97	38.0	71.0
Linseed oil meal	79	97	27.2	71.8
Peanut oil meal	83	98	38.7	75.7
Peanut oil meal (pressed)	90	96	38.4	74.7
Soybean oil meal (extracted)	97	96	40.7	73.0

Daniels and Nichols (19) 1917, found that the protein of the soybean is adequate for maintenance, growth and reproduction. This implies reasonable amounts of at least lysine and tryptophane.

The United States Department of Agriculture (97) 1917 says that "the value of soybean meal as a stock feed for producing meat, milk and butter is well established by practical experience supplemented by carefully conducted experiments made in both Europe and America".

Hays (37) 1919, concluded that "pigs gained most rapidly on coconut oil meal, next to this was soybean meal, oil meal stood third, and tankage fomrth". He stated further that "soybean meal proved to be the most economical".

Williams (101) 1919, reported that "in feeding trials with young chicks at the Pender test farm they found that when soybean meal was fed in equal quantities with wheat shorts and cracked corn meal with sweet milk, the soybean meal proved to be a most valuable feed and was found to be equal in value in the ration to rolled oats as a growth producer".

More recent work has in general borne out the earlier findings regarding the feeding value of soybeans. Huang (46) 1931 found that "soybeans make an excellent protein feed in the rations of brood sows", but that "soybeans are unsatisfactory for pigs in dry lot". According to Salmon (80) 1933, "linseed oil meal and soybean oil meal show practically equal values for fattening steer calves".

Morris and Wright (65) 1933, found from trials with producing dairy cows that the body uses bean meal more efficiently than either linseed or meat meal.

As has been indicated, the use of soybean flour has been confined largely to human consumption. The principle reasons for this limitation are (1) the cost of milling the flour, which extra cost adds nothing to its nutrient content, and (2) soybean products are used principally as protein supplements for feeding mature animals, for which uses the coarser grades serve as well.

Osborne and Mendel (70) 1917, reported from tests of certain proteins that lactalbumin is the best supplement to corn gluten of any tried but that in small replacements commercial milk albumin and soybean flour are equally affective.

Johns and Finks (50) 1921, from studies with replacing wheat flour with soybean flour in bread making, reported that "these mixtures of the soybean and wheat proteins were found two or three times more efficient than the proteins of wheat alone". They further concluded that "such a bread also contains an abundance of water-soluble vitamins".

Soybeans have formed a common article of human diet in the Orient for perhaps thousands of years. There the beans are prepared in various ways, one of the most interesting being the making of soybean "milk". According to Adolph and Wang (3) the origin of soybean "milk" is prehistoric. While slight variations in the method of preparing "milk" are reported by various writers, the following method given by Tso (94) 1928, outlines the essential steps in its manufacture. Regarding the making of soybean milk he stated: A measured quantity of yellow soybeans, partially milled to remove the seed coats, is soaked over night in a refrigerator in eight times its weight of tap water. The beans with the water are then put through a native stone mill to obtain a thin paste which is filtered through a fine-mesh horse-hair sieve. The yellowish white milky filtrate is the soybean milk". Tso gives the following as the average composition of the boiled milk:

Protein	4.4 per cent	Calcium	0.018 per cent
Fat	1.8 " "	Phosphorous	0.057 " "
Carbohydrate	1.5 " "	Total ash	0.410 " "

The first mention of soybean "milk" in America was made by Rurah (79) 1910, who reported the perfecting of a gruel flour for use in infant feeding.

The use of soybean milk has become so general in sections of the Orient that commercial plants have been established to manufacture and distribute the bottled product in much the same way that cow's milk is distributed in America. Adolph and Wang (3) reported in 1929 that soybean milk factories were located throughout China, and that the price of soybean milk in China averaged half the price of cow's milk. That factor alone would do much toward making the product popular among the poorer classes.

Since the first report by Rurah in 1910 of the manufacture of a gruel flour made of soybeans, the tendency in America has been to produce a soybean milk by simply mixing gruel flour in water and in most cases boiling the mixture. According to Piper and Morse (73) 1923, "experiments with different methods of preparing the milk have shown that as much bean curd (coagulated soybean milk) can be obtained by use of the meal as with cooking the beans and crushing, the latter being the method used in the Orient". Rurah's work aroused the interest of physicians over the country in the possibilities of soybean gruel as an infant food in cases of milk idiosyncrasy, and for adults in cases of diabetes. He reported (78) later (1915) that "these mixtures of condensed milk and soybean gruels will be found one of the most valuable additions to the dietary of the infant". He recommended their use in cases "when there is some question concerning the milk supply and then in instances where the infant is found to be incapable of digesting cow's milk. It permits a perfectly normal development as far as bones are concerned, and in successful feeding the infants present the appearance of breast-fed babies".

Sinclair (88) 1916, observed seventy-four infants who were fed soybean gruel because of gastro-intestinal disturbances. He stated in part that its use "has proved useful when mixed with cereals, oatmeal, or barley jelly. It may be used in broths".

In 1917 Park (71) reported that "when finely ground soybeans are mixed with about 10 parts of water and heated near the boiling point for 15 to 30 minutes, an emulsion is obtained which is remarkably similar in appearance and properties to cow's milk". This method of making soybean "milk" is an attempt to imitate the method of the Orientals.

Adolph (1) 1922, commenting upon the value of soybean milk, stated "Ladd has invented a homogenizing machine for the homogenizing of vegetable oils for difficult infant feeding. It would seem however, that the Chinese soybean milk contains such an oil already emulsified".

Metabolism trials with human subjects were carried out by Neuman (68) 1928. With bread made from a mixture of rye-wheat flour and soybean flour, the latter comprising 20 per cent of the bread, and such bread being the sole diet with the exception of water, he found the nitrogen balance remained positive until after seven diet periods of four days each, then there was a slight negative balance and a loss of 1.6 kg. of body weight. He concluded that "soybean bread is less well utilized than rye-wheat bread".

Despite the fact that the soybean and even soybean milk have been used for human food in the Orient for hundreds of years, it was apparently several years after the announcement of a gruel flour from soybeans, by Rurah in 1910, and his recommendation of it for infant feeding in abnormal cases, that the Orientals generally conceived the idea of its use in normal infant feeding.

Due probably to a lack of research workers in the Orient, the first controlled experiments upon the use of soybean milk in normal infant feeding were reported in 1928, eighteen years after Rurah had announced a soybean gruel flour, in America. Tso (94) fed a Chinese baby from the age of six weeks for a period of eight months on a diet consisting chiefly of soybean milk in amounts of from 600 to 880 cc. daily, supplemented by 50 to 70 gm. of cane sugar and 0.1 per cent of sodium chloride and from time to time by cod-liver oil, orange juice, egg, spinach, etc. During the experimental period the child increased 20.7 cm. in length and gained 4.1 kg. in weight, the growth even comparing very favorably with American standards. He concluded that "properly supplemented, it can be more or less comparable to cow's milk in nutritive properties".

Tso, Yee and Chen (96), used two infants 3-1/2 and 6 months of age at the beginning of the experiment, receiving soybean milk as their principle food, in metabolism trials and found that nitrogen balances were positive at all times.

Tso's (93) 1929, trials on the protein and mineral content of soybean milk, together with its contents of vitamins A and B showed that "at a level of 22 per cent the soybean milk proteins were slightly superior and at 14 per cent inferior, to those of cow's milk fed at a level of 11 per cent". He noted further that "the mineral content of soybean milk is inferior to cow's milk, but it is thought that the addition of a calcium salt and common salt would make up for this deficiency".

Chang and Tso (16) 1931, used a spray process for drying soybean milk and concluded upon the basis of a feeding trial with an infant for a period of 84 days, that "the dried soybean milk preparation resulted in growth at a rate superior to the standard weight curves for healthy breast-fed infants".

Tso and Chu (95) continuing the metabolism trials with infants first reported in 1928, confirmed their earlier conclusions and estimated that "approximately 80 per cent of the ingested nitrogen of soybean milk and 95 per cent of that of cow's milk are utilized".

Wan (98) 1931, concluded from rat-feeding experiments, that "soybean milk is poorer in vitamin A, but richer in vitamin B than cow's milk. The soybean milk was also deficient in vitamin D".

Siddall and Chiu (87) 1931, observed a Chinese baby which was fed on soybean milk, supplemented with rice porridge, vegetables, eggs and cod-liver oil, for 13 months and three weeks, from the age of 6 weeks. They found that at the age of 15 months he weighed 10 kg. and measured 75 cm. in length.

Although Chinese workers have had greater freedom in experimenting with human subjects in testing the merits of soybean food products, workers in this country have not been idle. Since 1928, the date of Tso's first report on the use of soybean milk in infant feeding, several investigators have reported work on this product. In 1921, Hill and Stuart (40) announced a "soybean food preparation for feeding infants with milk idiosyncrasy". Their (41) 1931 statement concerning this food preparation is that "soybean flour was taken as the source of the protein because it is rich in protein and because it has been carefully studied by a number of workers, who have found that it contains the correct amino acids for growth. The result of these studies indicates that it can be used as the sole source of protein in an infant's diet ---". They also said that "the bowel movements are likely to be somewhat looser in consistency than would be the case with a milk diet, but there is rarely a diarrhea ---".

Rittinger and Dembo (77) 1932 reported a comparison of the amino acid content of soybean milk with casein, as shown in table VIII. They found in measuring the vitamin D potency of soybean flour that 1/10 gram of powder satisfied a Steenbock unit. During the same year (1932) Ch'en and Adolph (17) reported that "cow's milk is very decidedly superior to soybean milk as a rickets preventative".

Wan (99) 1932, reported work upon the vitamin B_1 and B_2 contents of dried soybeans as compared to dried cow's milk (Klim), and concluded that "the soybeans are richer in B_1 than in B_2 and that the reverse is true of milk". He found that "soybeans contain only two-thirds as much B_2 but three times as much B_1, as Elim'.

TABLE VIII

AMINO ACID CONTENTS OF SOYBEAN MILK AND CASEIN

Amino acid	Casein	Soybean protein
	%	%
Alanine	1.85	not isolated
Valine	7.93	0.63
Leucine	9.70	8.45
Proline	7.64	3.78
Phenylalanine	3.88	3.86
Aspartic acid	1.77	3.98
Glutamic acid	21.77	19.46
Serine	0.50	not isolated
Tyrosine	4.50	1.86
Arginine	3.81	5.12
Histidine	2.50	1.39
Lysine	7.62	2.70
Ammonia	1.61	2.56
Tryptophane	present	present
Glycine	0.45	1.00
Cystine	present	present

Stearns (92) 1933, after studying the excretion and retention of nitrogen, calcium and phosphorous of an infant fed milk and various soybean preparations concluded that "the modified soybean food, from our observations, appears to be a satisfactory food for infants".

Klein (52) 1933, reported the healing of two cases of seborrheic eczema on a soybean flour diet. It would appear that under American conditions the most important use of soybean products in human nutrition would be in the abnormal or pathologic dietary.

Digestion experiments both in vitro and in vivo with soybean milk were reported by Adolph and Wang (2) 1934. They found in peptic digestion in vitro that at a pH of 1.66, 57.6 per cent of the soybean milk was digested in four hours, while at a pH of 1.67, 37.8 per cent of cow's milk was digested in an equal time. In tryptic digestion in vitro they found that at a pH of 10.25, 61.0 per cent of the soybean milk was digested in four and a half hours, while in the same time at a pH of 11.34, 78 per cent of the cow's milk was digested. They concluded that "when acted upon by both enzymes, the protein of cow milk is shown to be the more completely digested".

Reid (76) 1935, reported a soybean-egg powder which he prepared by suspending fine soybean flour in water, adding liquid egg-yolk and other supplements and spray-drying the mixture. The powder, according to Reid, forms an emulsion in water. This material produced a more rapid growth rate with rats than that obtained from the whole milk powder. Also "the haemoglobin building properties of the soybean-egg powder were found to be adequate for the rat".

MECHANISM AND CONTROL OF GASTRIC SECRETION

Digestion in the stomach is largely a function of the gastric juice which is secreted by the gastric glands, located in the mucosa lining the stomach. These glands are found more or less throughout the gastric mucosa but are much more numerous in the fundic region or along the greater curvature of the stomach. Moreover those glands located in other parts of the stomach secrete largely mucus and not the active digestive agents of the gastric juice. The glands are of two classes according to their location, viz. fundic and pyloric glands. The pyloric glands are said to be of the mucoid type and as such, have no concern with enzymatic digestion. In the fundic glands at least two types of cells are found (45), the chief or central cells, and the parietal or border cells. The latter are thought to be responsible for the hydrochloric acid of the gastric juice, while the former elaborate the enzymes and are sometimes spoken of as the "peptic" cells.

The gastric juice when secreted, contains at least three active ingredients, viz., rennin, which functions to coagulate milk in the stomach of the young animal; pepsin, which acts upon protein material, and hydrochloric acid, which serves to activate pepsin and to maintain an acid reaction of the stomach contents and thus prevent certain undesirable bacterial action in the stomach.

According to Halliburton (35), the secretory activity of the gastric glands is under nervous control for he stated that "as long ago as 1852 Bidder and Schmidt showed in a dog with a gastric fistula that the sight of food caused a secretion of gastric juice; and in 1878 Richet observed that in a man with complete occlusion of the gullet the act of mastication caused a copious flow of gastric juice". The early attempts however, to discover the secretory nerves of the stomach were not successful, according to Halliburton, so that it remained for the Russian physiologist, Pavlov, with a new technique, to actually demonstrate the location of secretory fibers to the stomach. He showed (72) not only that the secretion is under the control of the nervous system, but that the secretory fibers are carried in the vagus nerves. He demonstrated also that the presence of food in the mouth causes gastric secretion and designated this as "psychic secretion". He concluded further that secretion followed the placing of food in the stomach and also that the presence of digested food products in the intestines causes gastric secretion.

From a review of the literature dealing with psychic secretion, Alvarez (4) 1929, stated "some experimentors like Unber, Hertz and Sterling, Schrottenbach, Mantelli, Bogen, Sommerfeld, and Cade and Latarjet, working with men, women and children with good appetites, found little difficulty in securing gastric secretion when they allowed the subject to see, smell or taste food, or simply when they brought before him mental pictures of it; while Carlson with his more plegmatic Mr. V, found that food had to be tasted or chewed before a reflex secretion could be obtained". Dukes (20) stated that "the psychic phase of secretion is probably absent in ruminants". Mortenson (66) 1932, stated that "it seems entirely plausible that animals because of a less complex nervous system would be affected to a lesser extent than man by psychic conditions".

The second or gastric phase of gastric secretion was demonstrated by Pavlov to be due to the presence of food in the stomach. He showed that foods vary in their ability to excite the flow of gastric juice and that even water has the capacity to arouse slight secretion. He felt that the secretion resulting from the presence of food in the stomach is due entirely to secretagogues contained in the food, while Carlson (15) reported that secretion may be produced by mechanical irritation of the mucosa of the stomach.

The third or intestional phase of gastric secretion cannot be ascribed wholly to reflex activity since it may be made to occur after the vagi are severed. This was demonstrated by Ivy and Farrell (49) who transplanted a denervated stomach pouch into a mammary gland and found that gastric secretion occurred in the pouch about three to four hours after the animal had been fed. This indicates that the stimulus for the secretion just noted was something carried in the blood. Moreover, mechanical stimulation is eliminated in this case since the pouch was empty. It did not prove however that mechanical stimulation is not a factor in normal gastric secretion. Edkins (24) 1906, considered that the third phase of secretion is due to secretagogues in the food or food decomposition, which are absorbed into the blood where they effect the stimulation. The substance (secretagogue) which stimulates gastric secretion, he called "gastrin" or "gastric secretin".

Bayliss and Starling (7) 1902, had described such a mechanism to account for pancreatic secretion. Later Starling called such substances "hormones".

Ivy and Farrell (49) concluded that "the evidence presented shows decisively that a humoral mechanism is one of the mechanisms concerned in the genesis of gastric secretion".

The volume of juice secreted by the gastric glands was found by Pavlov to vary with the amount and character of food present in the stomach (72), and to be practically nil when the stomach is empty, which apparent fact has been made of extensive use in studying gastric digestion. Carlson reported however that even in fasting there is a small continuous secretion, but that during the act of eating and throughout the period of gastric digestion the rate of secretion is greatly increased.

Babkin (6) 1932, reported that at least in dogs, gastric secretion is an intermittent process. He insisted that any indication of a continual activity on the part of the gastric glands is likely the result of abnormal conditions.

Recent work has shown that various conditions can influence the rate of digestion in the stomach. Espe and Dye (28) 1932 found that during the third thirty-minute period a dog digested milk most rapidly as measured by the volume of gastric secretion, and that at the end of three hours the process of digestion in the stomach was complete. They concluded, regarding the effect of curd tension upon the time required for digestion that "doubling the curd tension of milk increases the length of the digestion period from 30 to 65 per cent".

Necheles, Sapoznik, Arens and Meyer (67) 1934, found that adding small amounts of hog's mucin, okra, olive oil and agar to a meal, influenced the rate of evacuation of the stomach. They reported that "at the beginning of digestion, hog's mucin increases and okra decreases gastric motility. After three hours time this is reversed and okra diminishes the emptying by 26 per cent". Their work involved both dogs and human subjects.

Northrop (69) 1919 reported that acetic acid diminished the rate of digestion of egg albumin, blood albumin, casein, and edestin but did not alter the rate of digestion of gelatin.

Webster and Armour (100) 1933 reported experiments which "indicate that the vitamins contained in yeast are necessary for the normal secretory activity of the gastric mucosa".

Dukes (20) 1930, quoted Alvarez as saying that "anything that stimulates the stomach will cause it to empty faster, while anything that stimulates the upper part of the intestine will delay its emptying".

Smith and Cowgill (90) 1933, reported from work upon proteins as stimulents for the secretion of pepsin, that "Pavlov's statement, that the quantity of enzyme produced is proportional to the amount and character of the nitrogen present in the diet is not confirmed by these observations made with different proteins as the source of nitrogen".

Belgoski (8) 1912, reported that the "gastric secretions from sweet milk, sour milk, hay and grass are near one another in value, and while the various kinds of feed differ in their characteristics, it is concluded that, in all probability other conditions besides the kind of feed affect the gastric secretion in ruminants". He reported further, as a result of his work with Pavlov pouches in calves that "the amount of the gastric juice of the ruminant and the free hydrochloric acid, depend apparently more upon the method of preparation of the food than upon the character of the latter".

Mortenson (66), in discussing the digestion of milk in the stomach as measured by palpating the abomasum through rumen fistulae reported considerable variation in the time of digestion, stating that "this variation is probably due to such factors as fatigue, individuality and state of health".

He found that milk which had been heated required less time for gastric digestion than raw milk.

Prytz (74) 1931, found from feeding trials with calves that "there is an indication that milk with hard curd regardless of fat percentage promotes a slightly better gain in kg. body weight than does milk with soft curd. There is an indication that milk with hard curd regardless of fat percentage is slightly better utilized than is milk with soft curd".

Hargrave (36) 1933, found that "the addition of butterfat to skimmilk decreased the length of time required for the milk curds to pass from the stomach. The average of the nineteen trials shows that the greater part of the skimmilk coagulum was liquified and passed from the stomach in 325 minutes whereas the coagulum from the three and six per cent milks passed from the stomach in 281 and 239 minutes respectively".

Espe and Cannon (27) 1935, in summarizing work to determine the effect of fat content in the milk upon the time required for its evacuation from the stomach, stated "curd from milk containing up to six per cent fat tends to leave the stomach more rapidly than skimmed milk due to the difference in texture of the curd formed. Milk containing this amount of fat does not apparently inhibit gastric secretion or gastric motility. Fat, in addition to being a valuable source of food, would appear to aid digestion when incorporated in the milk in limited amounts".

STATEMENT OF THE PROBLEM

The object of this investigation was to determine the rate at which soybean flour, fed as a gruel, passes from the abomasum of the calf. The secretion of gastric juice, which was used as a means of determining the rate of passage of the soybean flour from the stomach, was measured by (1) determining the volume of pure gastric juice secreted in Pavlov pouches, and (2) by determining the acidity of the stomach contents by use of rumen fistulae.

EXPERIMENTAL

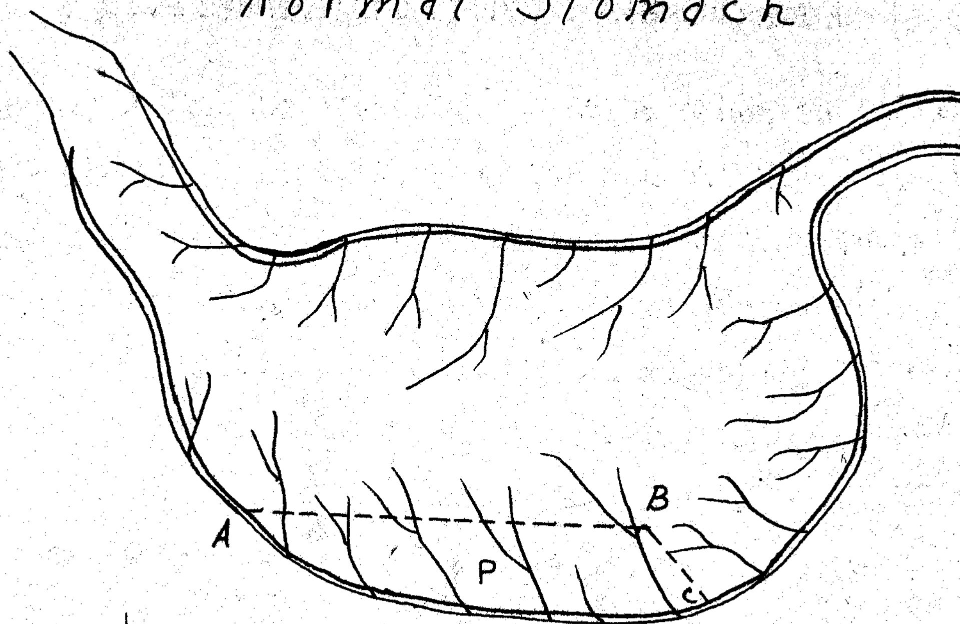
The original plan of this experiment was to determine gastric digestion of soybean flour by the exclusive use of palpation through rumen fistulae as a means of ascertaining the evacuation time of the stomach. This technique had been used with milk by Mortenson (66), Hargrave (36), and Espe and Cannon (27). The technique of palpation, however, while successful with milk due to the formation of a curd in the stomach by rennin, was useless for determining the evacuation time of a test meal of soybean flour, since no curd was formed with this material. The decision was then made to adopt the Pavlov pouch.

While the success of the Pavlov pouch as a means of measuring gastric secretion in dogs had been demonstrated, not only by Pavlov himself, but by numerous later workers, the applicability of this technique to ruminants has received very little attention. In fact, so far as it has been possible to glean from the literature, it had not been used in America prior to this investigation. In 1912, Belgoski (8) in Europe reported the successful use of the technique in calves while seven years earlier Grosser (33) had reported its use in goats. Accordingly a Pavlov pouch was established in one calf on January 30, 1934.

Technique of the Pavlov Pouch Operation

The operation to establish a Pavlov pouch in the calf does not differ materially from its establishment in single-stomached animals. The animal to be operated upon was fasted for 18-24 hours before the operation in order to allow the stomach time to empty itself before the anesthetic was given, since the effect of anesthetics is more positive if given when the digestive tract is reasonably free from food. The calf was then given chloral hydrate by means of a stomach tube, at the rate (usually) of 14 grams per 100 pounds of weight, as a general anesthetic. Some difficulty was encountered with the use of chloral hydrate because of the uncertainty of the exact amount necessary for complete anesthesia. The amount necessary for a particular calf seemed to be influenced by the general condition of the animal, the amount of fluid in the alimentary tract and the relative completeness and speed with which the material was absorbed, which was itself somewhat dependent upon the amount of liquid in the digestive tract. Some animals were lost from over-dosage while others of equal weight were not completely anesthetized when given the same dosage. When the animal was practically "under", it was placed upon an adjustable operating table (fig. 2), which had been designed to hold the calf on its back. In this position the animal was secured by ropes.

Normal Stomach



Stomach with Pavlov Pouch

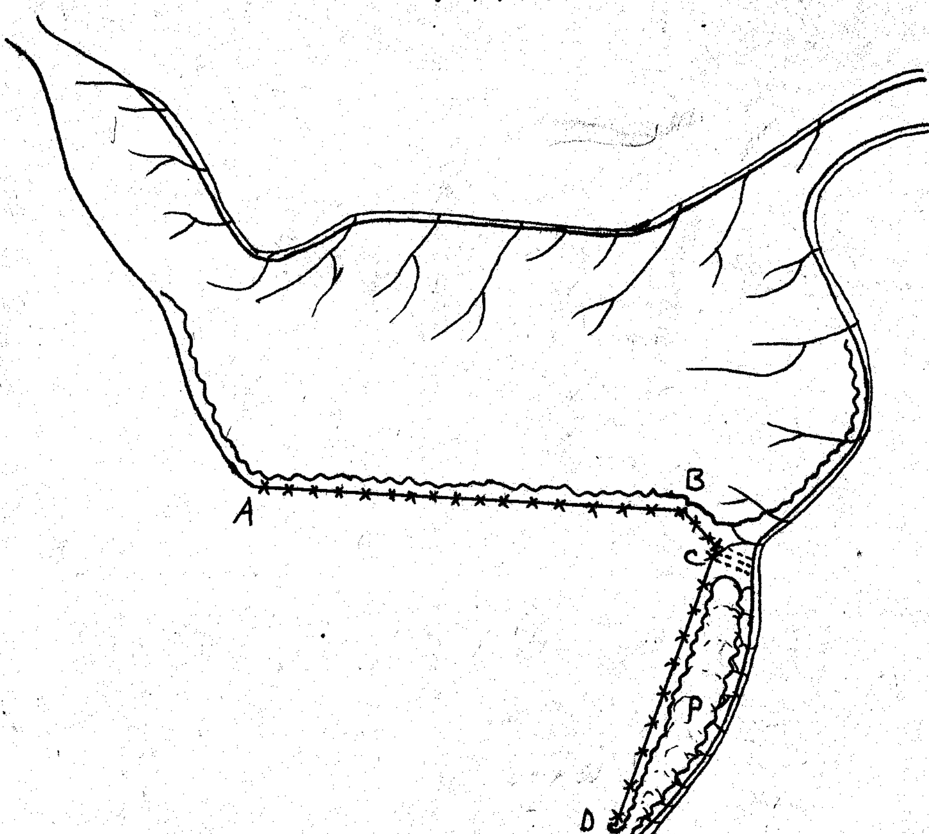


Fig. 2. Operating Table
Used in Pavlov Pouch Operation

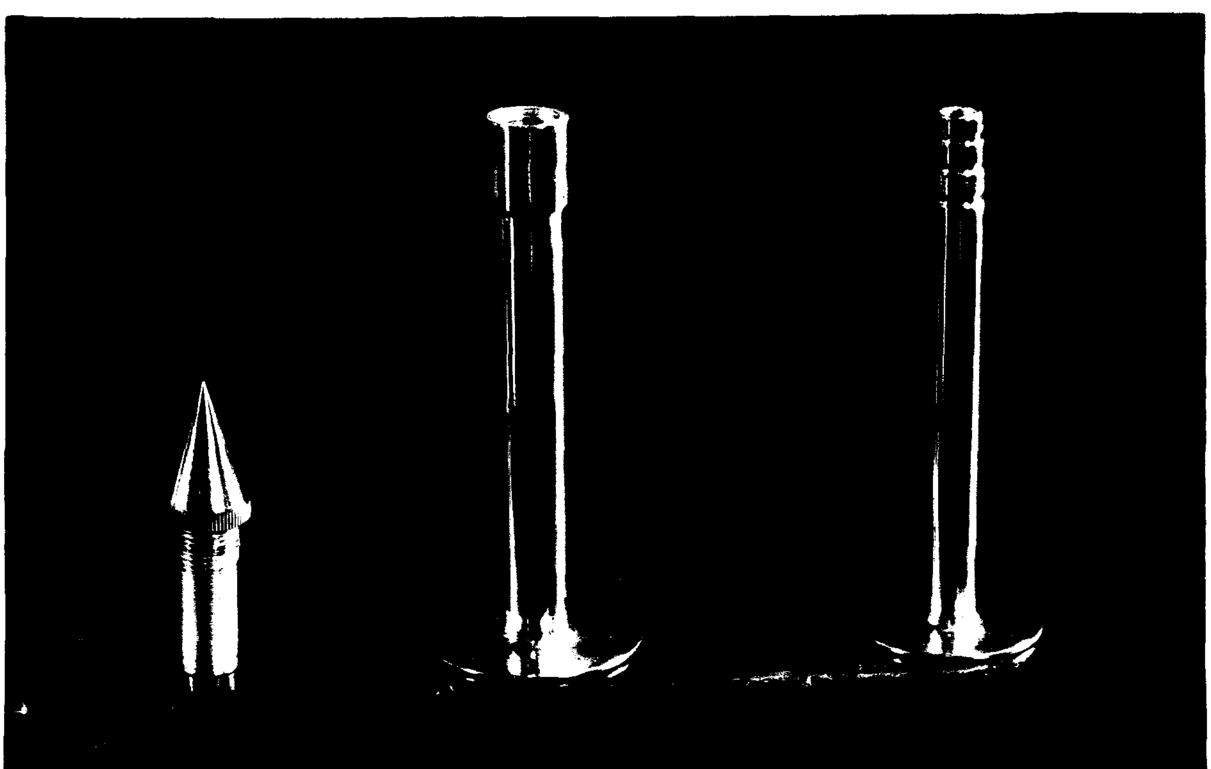


Fig. 3. Cannula
Used in Pavlov Pouches

The site for the incision was then smoothly shaved over an area sufficiently large to insure that no contact occurred between the incision and hair on the calf's body. The bare skin was then sterilized with tincture of iodine or iodine and glycerin and, during many of the operations the area was completely blocked by the injection of novocain over its various parts. This local anesthetic was more often omitted than used however since the completeness of the general anesthesia made the local anesthetic unnecessary. The animal was then completely covered with sterilized outing blankets.

An incision was made about two inches to the right of, and parallel to, the median, at such a point that after being extended forward for about three and one-half inches it ended near the breast bone. The abomasum was then drawn to the exterior and allowed to rest upon an electrically heated pad. An incision was then made through the abomasal wall in the fundic region starting at a point in the posterior-ventral portion of the stomach and running forward to a point just short of severing the ventral blood and nerve supplies to the organ, which follow the greater curvature of the stomach (fig. 1). This incision was made only after two clamps had been placed in such a position on the stomach that the incision came between them. These clamps served the triple purpose of practically closing the severed blood vessels, thus preventing excess bleeding; of holding the two parts of the divided stomach closed so as to prevent undue danger of infection from stomach contents, and finally of offering an anchorage in handling the stomach parts for suturing.

The stomach was then sutured, as was also the pouch or "pickle", by sewing first the mucosa layer and then the muscle layers, care being taken to maintain conditions as nearly aseptic as possible. Sterile saline at body temperature was used freely to prevent blood clotting on the stomach surface and to free the injured parts of contamination by stomach contents.

In the early operations the pouch was brought to the outside through a second incision in the abdominal wall (after the remaining stomach had been replaced), and secured by suturing to the skin around this second incision in such a way as to leave the lumen of the pickle open to the outside. In later operations however, a modification of the cannula designed by Dragstedt (fig. 3), for use in human gastric feeding, was used. The use of this cannula simply required closing completely the lumen of the pickle, after the cannula had been placed through a side of the pouch wall, making of the pouch, a blind sac. The omentum of the stomach was then wrapped and secured about the cannula stem in order to protect the incision through the pouch wall. The cannula was then brought to the exterior through a stab incision made by a sharpened, removable point on the cannula (fig. 3 left). This technique proved much more successful than the older method of suturing the pouch to the abdominal wall, used by Pavlov and others, since it required no dressing after healing and moreover the cannula always conducted the secretion away from the skin surface and thus the need for Fuller's earth packs to prevent digestion of the skin by the gastric juice, was dispensed.

The original incision in the abdominal wall was then sutured, first the peritoneum, then the muscle wall and finally the skin. A pack was kept over the incision for some days to prevent infection from stall bedding and etc. In some of the later operations lint dressings were plastered over the incision and allowed to remain until such time as the stitches were removed.

The early operations in 1934 were as a whole more successful than the later ones of the same year despite the better technique employed in the later ones, due to the fact that they were performed during cold weather with a minimum danger of infection while the later ones were done in very warm weather (June), which offered optimum conditions for infection. Very little infection occurred during the cold weather but considerable losses were suffered from those operations performed later in the season. Again in 1935, those operations done early (August) were less successful than those performed later (October), the later ones showing little tendency for infection.

Technique of the Rumen Fistula Operation

In order to permit feeding directly into the stomach in such a way as to overcome any effects of rumen activity, and also to permit the determination of the acidity of gastric contents, fistulae were placed in a number of calves. In two cases fistulae were established in calves in addition to Pavlov pouches which had been previously established. In establishing the fistulae, the technique of Schalk and Amadon (84) was employed. The surgery involved in this operation is less complicated than that necessary for the Pavlov pouch, since the fistula concerns only the rumen which in the two- or three-weeks old calf is only partially developed and normally is not functioning. The procedure however so far as anesthesia, antisepsis and etc., are concerned, is the same. The incision was made about three inches long diagonally across the triangle or "hunger hollow", and through the rumen wall. The rumen wall was then sutured to the skin in such a manner as to leave an opening from the rumen to the outside (fig. 4). The muscle layer just under the skin was left open. These incisions extended for a length of about three inches, but when healing was complete, a process of stretching was begun by the use of plugs of varying sizes (fig. 5), larger plugs being used as the walls relaxed until the incision would easily admit the hand and arm (fig. 6). During the healing

Fig. 4. Rumen Fistula Showing Method of Feeding Test Meals. Calf 47Y.

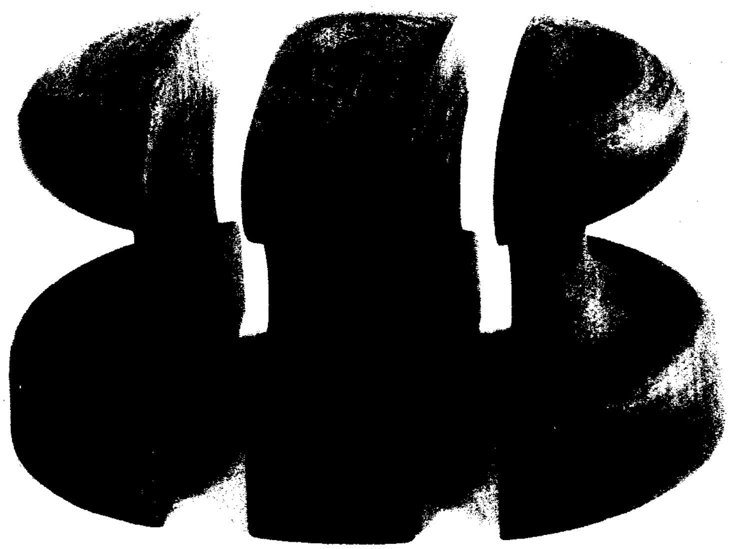


Fig. 5. Wooden Plug Used in Closing Fistula Opening.

Fig. 6. Close-up View of Fistula Opening. Calf 47A13.

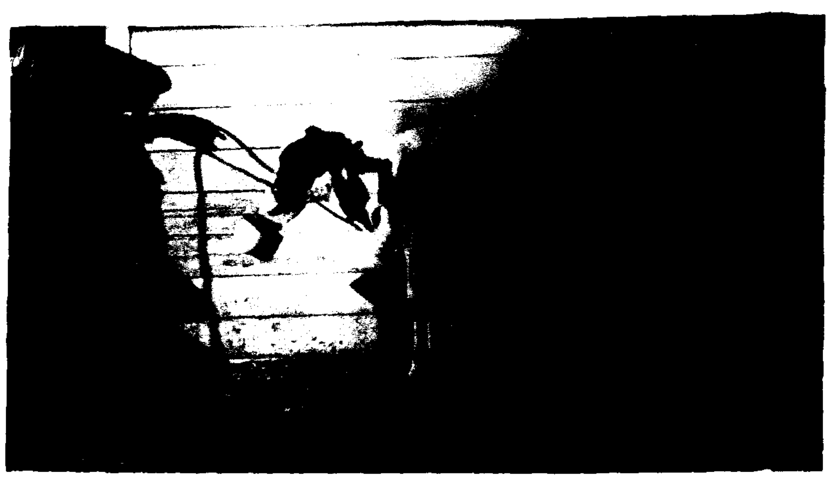


Fig. 7. Showing Arrangement of Rehfuss Tube for Taking Acidity Samples. Calf 1306.

Fig. 8. Close-up of Cannula and Balloon Showing Method of Collecting Gastric Juice. Calf 47A12.

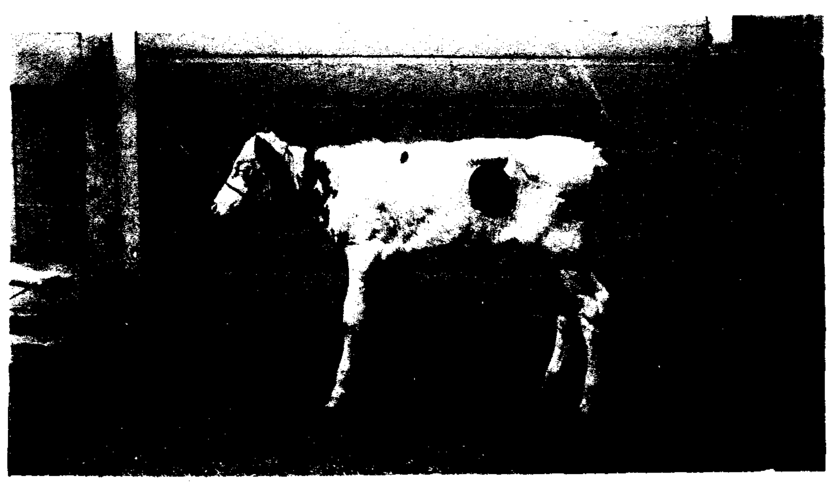


Fig. 9. 47Y With Both Pavlov Pouch and Rumen Fistula.

process, the rumen was packed through the opening, with muslin, which absorbed the liquid rumen material and thus prevented its escape through the opening, which would aggravate the healing process.

With animals in which it was determined to place both the Pavlov pouch and the rumen fistula, the operations were performed in the order named as the losses sustained were largely the result of the pouch operation.

A week to ten days after either operation the animal was usually ready for experiment. Some calves were able to recover from either operation in six or eight days while others required two to four weeks. They seemed very susceptible to pneumonia while recovering from the effects of the operations so that extreme care was exercised to prevent unnecessary exposure during this period.

Experiments With Calves Having Pavlov Pouches and Rumen Fistulae

The investigations involving the gastric secretion produced by soybean milk as compared to cow's milk were conducted in three parts as follows:

1. A series of twelve-hour trials using a test meal of one liter of soybean milk or whole cow's milk, fed after a fasting period of 24 hours, with the volume of gastric secretion from the Pavlov pouch being determined at half-

hour intervals.

2. A continuous trial of 14 days' duration involving soybean milk and whole cow's milk, with regular feeding each eight hours and readings of the volume of gastric secretion in the Pavlov pouch being made at half-hour intervals throughout the fourteen days - day and night. Three to four pounds of soybean milk each eight hours comprised the diet during the first seven days and the same amount of whole cow's milk made up the ration for the second seven days.

3. A series of sixteen-hour trials involving (a) the feeding of a test meal of one liter of skimmed cow's milk or one-half liter of "fortified soybean milk", (described below), after a fasting period of twelve hours, preceded by twelve hours on oatmeal gruel, to calves with Pavlov pouches, with the volume of gastric secretion in the Pavlov pouches being determined at half-hour intervals, and (b) the feeding of similar test meals to calves with rumen fistulae, with the free and total titratable acidity being determined in samples taken hourly from the true stomach.

The soybean "milk" used in series 1 and 2 was made by stirring soybean flour into warm water at the rate of one part of flour to nine parts (by weight) of water. The soybean milk was fed in the same manner as cow's milk. The following figures are given as the nutrient content of this soybean milk, based on Henry and Morrison's figures for soybean oil meal:

Protein	3.97 per cent
Carbohydrate	3.47 per cent
Fat	0.45 per cent

The soybean milk or gruel used in the third series was made of one-third fresh skimmilk (dry matter basis) and two-thirds soybean flour, with 4 cc. of a 40 per cent solution of calcium chloride per half-liter. This mixture is referred to subsequently as "fortified soybean milk".

The cow's milk used as a check in series 1 and 2, was Holstein milk taken from two cows in the college herd, numbers 975 and 903, with average curd tensions of 95 and 82 grams, and average fat tests of 3.0 and 2.69 per cent, respectively. Whole milk from number 975 was used in the twelve-hour trials, whole milk from number 903 was used in the continuous trials, and skimmilk from number 975 was used in the sixteen-hour series, both as a check meal and as the solvent for the soybean gruel.

Twelve-Hour Trials

These trials were started on February 7, 1934, and continued at intervals until June 6, 1934, with a total of 28 trials or days on cow's milk and 14 days on soybean milk, or a total of 42 trials. In each trial 1 liter of feed was given at the end of a 24-hour fast.

The first calf, 47N, was prepared with the original sutured type of pouch mentioned above. This calf was run for three trials on cow's milk, but was not used for any soybean trials, so that no comparison could be made. The results of these trials, therefore, were not recorded. This calf died February 20.

The trials were resumed on April 4, with four calves, numbers 47U, 47Y, 47A1, and 47A2, as shown in the following table:

TABLE IX

SUMMARY OF 12-HOUR TRIALS (DAYS), WITH PAVLOV POUCH CALVES FED WHOLE COW'S MILK AND SOYBEAN MILK

Calf No.	Cow's milk	(days)	Soybean Milk	(days)
47U	(mouth fed)	9	(mouth fed)	3
47A1	(mouth fed)	8	(mouth fed)	3
47Y	(mouth fed)	9	(mouth fed)	5
47A2	(fistula fed)	2	(fistula fed)	3
Total		28		14

These calves were approximately six weeks old at the beginning of this series. They were kept in individual pens in the experimental barn at the college dairy farm, and bedded on wood shavings. Muzzles were kept on the calves except at feeding time. Between trials they were fed three times daily. Only milk was fed during these periods between trials.

Calf 47A1 died of pneumonia caused from a fistula operation on June 1, and 47U died from the same cause on June 29.

Calves 47Y and 47A2 were subjected successfully to a second operation in which rumen fistulae were established. With calf 47A2 the test meals were placed directly in the true stomach by means of a tube. The trials marked "fistula fed" in table IX, were conducted in this way.

Immediately following the feeding of the test meal, a rubber balloon with a piece of glass tubing and rubber stopper secured in one end, was placed over the tip of the cannula (fig. 8) and secured in place by means of a rubber band. Readings were taken each half-hour, the volume of gastric juice being measured in a graduated cylinder.

Some difficulty was encountered from trapping of the juice in the folds of the gastric mucosa. For example quite often an unusually small reading would be followed by an unusually large one. When the two readings were averaged it would be found that the calculated readings were almost always in line with other readings near them. This condition was unquestionably trapping.

Various methods were tried for preventing this occurrence, some of which were partially successful. In later operations, light, spider-shaped guards were welded on the tops of the cannulae before the latter were placed in the pouches. These guards aided greatly in preventing the mucosa folds from closing the cannulae outlets. Again sometimes trapped juice was released by gently passing a glass tube, rounded on the end, into the cannula in such a way as to lift the mucosa fold from obstructing the opening. In tabulating the readings in single trials, wherever a case of obvious trapping occurred the two readings concerned were averaged, which procedure did not alter the total secretion but which on the other hand, more nearly revealed the actual conditions of secretion.

Continuous Trials

This series of trials was started July 15, and continued for fourteen days, ending on July 28. The first seven days (24-hour periods) were used for soybean milk, while the second seven days were used for whole cow's milk. These trials are summarized in table X below:

TABLE X

SUMMARY OF DAYS OF CONTINUOUS SERIES. EACH 24-HOUR PERIOD IS CONSIDERED ONE DAY

Calf No.	Days, cow's milk	Days, soybean milk
47Y	5	6
47A2	7	7
47A11	7	3
47A12	7	7
Total	26	23

For this series calves 47Y and 47A2 (previously mentioned) were used as a "fistula group", while two other calves, 47A11 and 47A12 were prepared with pouches only, and used as a "mouth-fed group". These calves were bedded and cared for in the same way as those in the 12-hour trials. Readings of the volume of gastric secretion were taken at half-hour intervals during the entire fourteen days - day and night. The calves were fed each eight hours so that each 24 hours really consisted of three eight-hour trials. Calves 47Y and 47A2 were fed, by way of fistula, four pounds each per feed, or twelve pounds each per day of either the check ration of whole milk or of the experimental ration of soybean milk (a 1:9 mixture of soybean flour and water). Calves 47A11 and 47A12, being younger, were fed three pounds each per feed or nine pounds each per day. The feeding hours were 5:00 a.m., 1:00 p.m., and 9:00 p.m.

A Carrier for Soybean Flour

Experience with the above groups of calves suggested the need for designing some method of forcing a greater percentage of the flour or gruel to remain in the stomach for a time sufficient for peptic activity, to prevent scouring which was apparently the result of the too rapid passage of the material through the alimentary tract. The tendency for calves to scour while being fed soybean gruel was demonstrated rather conclusively by the author (86), in a feeding trial in which calves fed soybean gruel remained quite loose throughout the trial. It is possible however that at least some of the looseness was due to the presence of a foreign protein in the digestive tract rather than a too rapid passage through the intestines. Since the gruel would not curdle with rennin the best prospect seemed the use of a portion of milk as a carrier.

Accordingly skimmilk was used as the solvent for the soybean flour in place of water, in the hope that the curd formed by the milk would hold the soybean flour so that it would be released only as the curd was digested. In vitro experiments however, revealed that milk, after having had soybean flour dissolved in it, lost its capacity for coagulation with rennet.

The next task was to restore to the milk its capacity for coagulation with rennet, after the addition of the soybean flour. Since the soybean flour is somewhat low in calcium, and since calcium is known to be an important factor in milk coagulation (28), it was felt that the inability of rennet to coagulate milk containing soybean flour was probably due to the fact that the calcium content of the milk, necessary for coagulation, was absorbed by the soybean flour. Should such be the case, the addition of a soluble calcium salt would be expected to replace this calcium and thus restore the coagulating ability. Accordingly calcium salts were tried and found to actually restore the coagulability of the milk. First calcium lactate and later calcium chloride, was tried, the latter being used in all of the in vivo trials. The calcium salt was found to enable coagulation to occur regardless of the amount of flour added, although the curd tension became less as the amount of flour was increased.

The Hill test (42) for curd tension was used as a means of determining the necessary dilution with milk, as well as the amount of calcium chloride necessary to effect a maximum coagulation. Skimmilk from cow number 975, whose curd test was 95 grams, was used as the solvent for the flour, and a mixture of 10 grams of flour in 100 cc. of skimmilk was taken as the standard in determining the amount of calcium chloride necessary. The data regarding the amount of calcium chloride necessary to insure coagulation are shown in tables XI, XII, XIII, XIV, XV, and XVI. It will be seen from these tables that the amount of calcium chloride necessary to produce a given curd tension varied, within limits, with the amount of flour used.

While the 30-gram sample (table XIII) had a curd tension of 52 grams, which would have been considered ample, it was so viscous that it would be very difficult for a calf to drink the mixture (see table XVII).

Because the curd tension of milk from "soft-curd cows" often runs as low as 20 grams, it was felt that the soybean flour might be increased in the mixture in proportion to cow's milk, above the point of 20 grams per 100 cc., provided some means of making the mix thinner so that the calves could drink

TABLE XI

SERIES TO DETERMINE AMOUNT OF A 25 PER CENT SOLUTION OF CALCIUM CHLORIDE EFFECTIVE IN HARDENING CURD

Content of sample	$CaCl_2$ cc.	Rennet cc.	Curd tension (gm)
Soybean flour 10 gm. Skimmilk 100 cc.	0.5	1	91
Soybean flour 10 gm. Skimmilk 100 cc.	0.6	1	90
Soybean flour 10 gm. Skimmilk 100 cc.	0.4	1	75
Soybean flour 15 gm. Skimmilk 100 cc.	0.5	1	62
Soybean flour 20 gm. Skimmilk 100 cc.	1.0	1	73
Soybean flour 20 gm. Skimmilk 100 cc.	0.5	1	no curd

TABLE XII

SERIES TO DETERMINE AMOUNT OF A 35 PER CENT SOLUTION OF CALCIUM CHLORIDE EFFECTIVE IN HARDENING CURD

Content of sample	$CaCl_2$ cc.	Rennet cc.	Curd tension (gm)
Soybean flour 10 gm. Skimmilk 100 cc.	0.5	1	90
Soybean flour 20 gm. Skimmilk 100 cc.	0.5	1	no curd
Soybean flour 20 gm. Skimmilk 100 cc.	1.0	1	79

it, could be devised. Water was then used to thin the mixture. Water was expected, of course, to weaken the curd tension rather

TABLE XIII

SERIES TO DETERMINE AMOUNT OF A 40 PER CENT SOLUTION OF CALCIUM CHLORIDE EFFECTIVE IN HARDENING CURD

Content of sample	$CaCl_2$ cc.	Rennet cc.	Curd tension (gm)
Soybean flour 10 gm. Skimmilk 100 cc.	0.3	1	84
Soybean flour 10 gm. Skimmilk 100 cc.	0.4	1	98
Soybean flour 10 gm. Skimmilk 100 cc.	0.5	1	105
Soybean flour 10 gm. Skimmilk 100 cc.	0.6	1	102
Soybean flour 15 gm. Skimmilk 100 cc.	0.75	1	86
Soybean flour 20 gm. Skimmilk 100 cc.	1.0	1	75
Soybean flour 30 gm. Skimmilk 100 cc.	1.5	1	52

rapidly. Espe and Dye (28) found that diluting milk with an equal amount of water resulted in a curd tension of 26 grams, while a dilution of two parts of water to one part of milk gave a curd tension of 1 gram. It was felt that a curd tension of 20-40 grams would be satisfactory for calf feeding, so that water and soybean flour might continue to displace milk in the mixture until this curd tension was reached. In table XIV are shown the data from water dilution at the rate of 1 part of water to 1 part of skimmilk.

Table XIV shows that a dilution of 1 part water to 1 part skimmilk gives a curd tension of approximately 25 grams when

TABLE XIV

SERIES TO DETERMINE MAXIMUM DILUTION WITH WATER AND SOYBEAN FLOUR WHICH WILL RETAIN IN SKIMMILK A CURD TENSION OF 20-40 GM.

Content of sample	$CaCl_2$ cc.	Rennet cc.	Curd tension (gm)
Soybean flour 20 gm. Skimmilk 50 cc. Water 50 cc.	1.0	1	25
Soybean flour 30 gm. Skimmilk 50 cc. Water 50 cc.	1.5	1	27

used with either 20 grams of flour per 100 cc. of solvent or with 30 grams. Thirty grams of flour in 100 cc. of solvent, again gives a mixture which is quite viscous (see table XVII), so that a calf would have difficulty in drinking it. The 20-gram sample, which gives a ratio between milk nutrients and soybean flour nutrients of 1:4 or 20 per cent milk, on a dry weight basis, was used in the remainder of the in vitro tests.

After the above mixture had been determined, a series of tests was run to determine the minimum amount of calcium chloride that would form a curd of as much as 20 grams with the mixture. These data are tabulated in table XV.

It will be seen from table XV that the addition of calcium chloride (40 per cent solution) at the rate of 0.9 cc. for a sample containing twenty grams of flour, or 0.45 cc. per ten grams of flour, suffices for a curd tension of 20 grams or more. The skimmilk used as a solvent had a curd tension of 95 grams.

Preliminary tests with calves, however, showed the 20 per cent (dry weight basis) mixture to be unpalatable. Accordingly the proportions were shifted so as to produce a mixture in which the dry matter of the skimmilk formed approximately one-third of the total dry matter of the ration. The total solids of this mixture were approximately twenty per cent. In table XVI are shown the make-up and curd tension of this mixture. This gruel

TABLE XV

SERIES TO DETERMINE MINIMUM 40 PER CENT CALCIUM CHLORIDE WHICH GIVES A CURD OF 20 GRAMS OR MORE IN A 50-50 MILK AND WATER DILUTION

Content of sample	$CaCl_2$ cc.	Rennet cc.	Curd tension (gm)
Soybean flour 20 gm. Skimmilk 50 cc. Water 50 cc.	0.8	1	15
Soybean flour 20 gm. Skimmilk 50 cc. Water 50 cc.	0.9	1	25
Soybean flour 20 gm. Skimmilk 50 cc. Water 50 cc.	0.9	1	25 (check)

in the calf's stomach did not produce a hard or rubbery curd as would have been expected from the in vitro tests, due probably to dilution with additional liquid in the stomach. For this reason the calcium chloride content was increased to 1 cc. per

TABLE XVI

MAKE-UP AND CURD TENSION OF SOYBEAN RATION CONTAINING ONE-THIRD SKIMMILK (DRY WEIGHT BASIS)

Content of sample	$CaCl_2$ cc.	Rennet cc.	Curd tension (gm)
Soybean flour 22 gm. Skimmilk 100 cc. Water 30 cc.	0.77	1	41
Soybean flour 22 gm. Skimmilk 100 cc. Water 30 cc.	1.00	1	51

22 gram sample (table XVI) in the hope of overcoming the effect of this dilution. The gruel then produced a very thick soup-like mass which remained in the stomach a sufficient time for peptic digestion.

In table XVII are summarized data concerning the mixtures used in the in vitro determinations of calcium chloride needed to restore a curd tension of 20-40 grams in the milk to which soybean flour and water had been added. Mixture number 7 represents the ration used in the in vivo tests.

Sixteen-Hour Trials

Following the preliminary twelve-hour, and continuous trials and after a mixture of soybean flour and milk, fortified with calcium chloride so that it would form a coagulum with rennet, was devised, an extended series of experiments

TABLE XVII

SUMMARY OF MIXTURES USED IN IN VITRO TESTS IN DETERMINING SOYBEAN FLOUR-SKIMMILK MIXTURE

Mix	Amount --			Dry matter		Dry matter			Maximum	
No.	Flour	Skimmilk	Water	Flour	Milk	Flour	Milk	Total	curd Tension	Viscosity (Scott
	gm.	cc.	cc.	gm.	gm.	%	%	%		
1	10	100		9.00	10.266	7.915	9.029	16.944	100	9.96
2	15	100		13.50	10.266	11.372	8.659	20.021	86	11.79
3	20	100		18.00	10.266	14.551	8.299	22.850	75	13.52
4	30	100		27.00	10.266	20.193	7.679	27.872	50	263.35
5	20	50	50	18.00	5,133	14.771	4.213	18.984	25	11.75
6	30	50	50	27.00	5.133	20.477	3.893	24.370	27	86.65
7	22	100	30	20.80	10.266	13.359	6.593	19.952	51	13.00

of sixteen hours' length, was inaugurated to determine gastric digestion in calves fed "fortified soybean milk", as measured by gastric secretion. These experiments were started on September 16, 1935 and continued at the rate of approximately two per week until November 23, 1935, with a total of 100 trials. Five calves with Pavlov pouches and three with rumen fistulae were used in this series. Of these eight, four were Holstein, three were Guernsey, and one was an Ayrshire. At the beginning of the trials the pouch calves ranged from four to nine weeks of age, the operations to establish Pavlov pouches having been performed when the animals were two to three weeks of age. The fistula calves were approximately ten months old at the beginning of the series. The rumen fistulae had been established in these calves some nine months previous to the beginning of these trials. All of the calves were kept muzzled except while being fed, and were allowed only milk and oatmeal gruel between trials. They were fed three times per day. Once each day, except on test days, cod-liver oil, mineral and yeast were given as protective foods.

Sixteen hours was chosen as the length of the trials because earlier experiments at this station (27) indicated that a test meal of one liter of skimmilk disappears from the calf's stomach on the average in seventeen hours. No milk was fed for a period of twenty-four hours preceding each experiment it being replaced by oatmeal gruel up to twelve hours before each trial began. It had been determined previously that oatmeal gruel leaves the stomach in less than twelve hours.

Fortified soybean milk, (table XVI), was checked against a ration of skimmilk, using skimmilk from the same batch as was used to dissolve the soybean flour. The experimental test meal consisted of 500 cc. of the "fortified soybean milk" and the test meal of the skimmilk check ration consisted of 1000 cc. The volume of the experimental test meal was cut to 500 cc. because its dry matter content was 20 per cent while that of skimmilk was of course approximately 10 per cent. All test meals were fed at body temperature.

a. Volume of Gastric Secretion

Five calves, numbers 1344, A30, A31, A32 and A34, prepared with Pavlov pouches were used in a series of sixteen-hour experiments to compare the volume of gastric juice secreted by a given pouch after a meal of soybean flour in milk, with the volume produced after a meal of skimmilk.

Immediately following the feeding of the test meal, rubber balloons (fig. 8), with pieces of glass tubing fitted with rubber stoppers (described earlier), were placed on the cannulae and readings taken at half-hour intervals throughout the sixteen-hour period, the volume of secretion being determined in the same manner as described earlier with the twelve-hour experiments. Table XVIII summarizes the number of experimental days in the sixteen-hour series with Pavlov pouch calves.

TABLE XVIII

SUMMARY OF SIXTEEN-HOUR EXPERIMENTS TO DETERMINE VOLUME OF GASTRIC JUICE SECRETED IN PAVLOV POUCHES

Calf No.	Skimmilk, days	Soybean milk, days
1344	6	8
A30	7	5
A31	4	5
A32	6	5
A34	5	5
Total	28	28

b. Acidity of Gastric Contents

Three calves, numbers 1306, A13 and A14, prepared with rumen fistulae, were used in a series of 44 trials distributed as in table XIX, in determining the acidity of the stomach contents at one hour intervals through the sixteen hours. With these calves the test meals were fed directly into the abomasum through the fistulae by means of an aspirator bottle and rubber tube, arranged in such a way as to deliver the meal into the stomach at a constant rate. Any water that was found in the rumen was removed before the test meals were given.

TABLE XIX

DISTRIBUTION OF SIXTEEN-HOUR TRIALS WITH RUMEN FISTULAE CALVES FED "FORTIFIED SOYBEAN MILK" AND SKIMMILK

Calf No.	Days on skimmilk	Days on soybean milk
1306	10	9
A13	10	8
A14	4	3
Total	24	20

Immediately after the meal was placed in the true stomach, a Rehfuss tube was extended through the reticulo-omasal and omaso-abomasal orifices in such a way that the tip rested on the floor of the abomasum. The other end of the tube was then placed through a hole in the fistula plug (figs. 5 and 7), and secured in this position by tying to an elastic band placed around the body of the calf. The hourly samples were taken by drawing small amounts (3-4 cc.) of stomach contents through the tube into a syringe. Each sample was immediately strained through cheese cloth to remove large particles, after which it was ready for titration. Free acid was determined by titration with N/100 sodium hydroxide, using Topfer's reagent (dimethylaminoazobenzine) for an indicator. Phenolphthalen was then added and the total acid determined by continued titration with this indicator.

Because of the relatively small amount of liquid in the stomach when the soybean meal was used, it was often necessary to draw samples of six to ten cubic centimeters in order to obtain enough strained material for titration. Also during the last three or four hours of the experimental period, it was often difficult to get any liquid material for titration, due to the fact that most of the meal had passed from the stomach.

DISCUSSION OF RESULTS

Because no method was known to accurately measure the volume of the pouches in the calves used, and also because wide variations are found in different calves as regards their gastric secretion, ranging from what may be termed "natural hypo-secretion" to "natural hyper-secretion", it was not possible to compare one calf with another in considering the results obtained in this investigation. Instead, the various trials with one calf on soybean flour were checked against the trials on cow's milk with the same animal. This rule was adhered to throughout the investigation.

Twelve-Hour Trials

The results of the trials listed in table IX, are shown graphically in figures 10, 11, 12, and 13. It will be recalled that in this series of trials the soybean ration consisted simply of soybean flour, stirred into warm water at the rate of one part flour to nine parts water, nothing having been added to aid coagulation in the stomach.

In the trials with calf 47A2 (fig. 13), which had a rumen fistula in addition to a Pavlov pouch, the test meals were placed directly in the abomasum by passing a tube through the reticulo-omasal and omaso-abomasal orifices and allowing the meal to flow from an aspirator bottle through the tube.

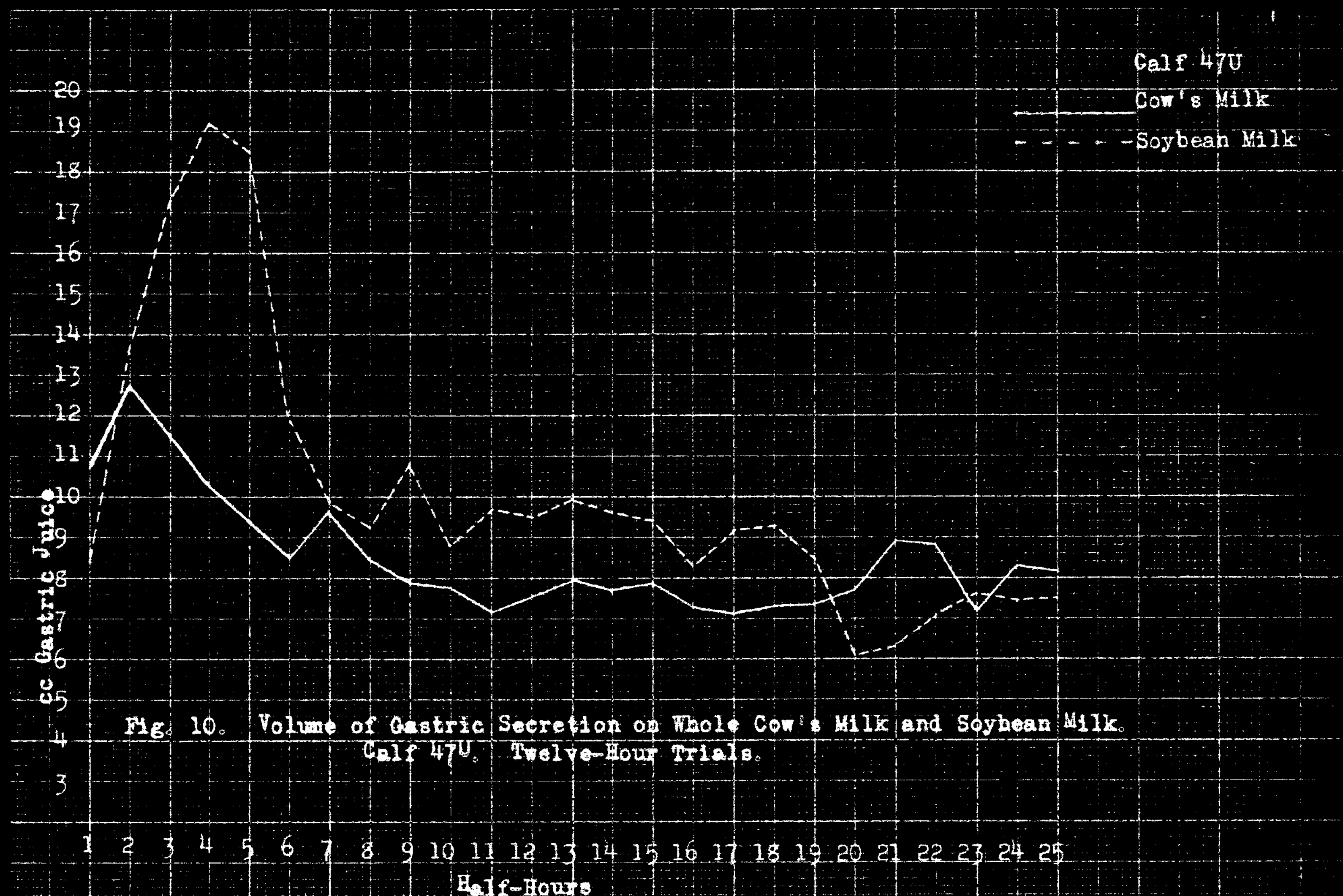


Fig. 10. Volume of Gastric Secretion on Whole Cow's Milk and Soybean Milk.
Calf 47U. Twelve-Hour Trials.

Fig. 11. Volume of Gastric Secretion on Whole Cow's Milk and Soybean Milk. Calf 47A1. Twelve-Hour Trials.

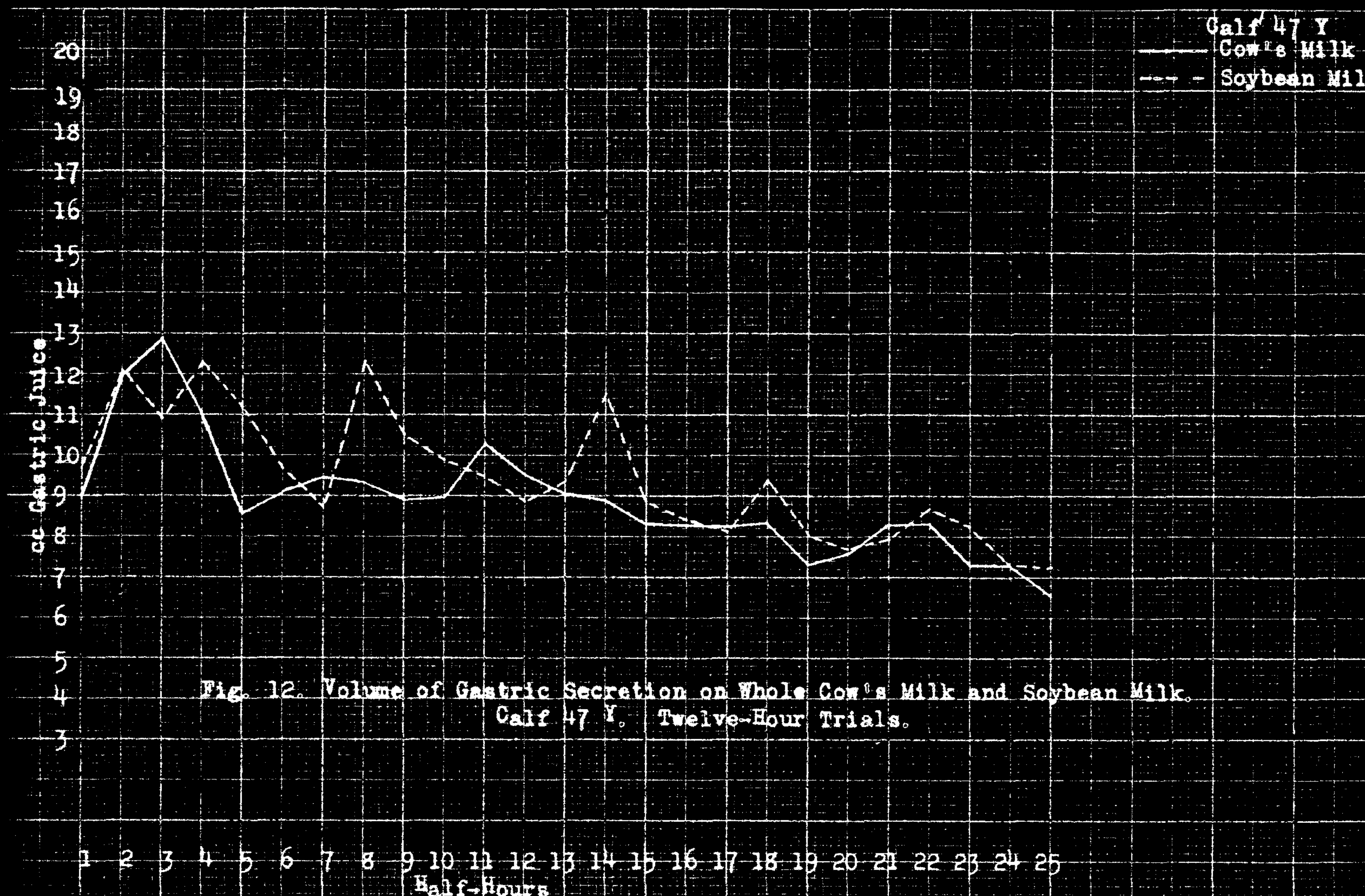


Fig. 12. Volume of Gastric Secretion on Whole Cow's Milk and Soybean Milk.
Calf 47 Y. Twelve-Hour Trials.

Fig. 13. Volume of Gastric Secretion on Whole Cow's Milk and Soybean Milk.
Calf 47A2. Twelve-Hour Trials.

It may have been that the influence of the first three stomachs was thereby eliminated. As may be seen however the results with this calf were similar to those with mouth-fed calves.

A glance at figures 10, 11, 12, and 13 shows that the volume of gastric secretion diminished from a point one and one-half hours after feeding, throughout the balance of the period during which measurements were taken, which in this series was twelve hours. The decrease is seen in both the soybean and the check trials. This decrease in the volume of gastric juice was by no means gradual, having fallen rapidly from the second hour to about the fourth hour, after which the decrease became much less rapid, and tended to plateau off, but finally fell to a volume of six or eight cubic centimeters per half-hour period at the end of the twelve hours. In no case did it reach zero.

While the same form of curves was produced by both the soybean milk and the cow's milk, certain differences are at once apparent. In the first place the total volume of gastric juice secreted during the twelve hours was greater by 11.87 per cent with the soybean ration than with cow's milk (table XX). Moreover the total volume secreted during the first six hours of the experimental period was greater by 21.41 per cent with soybean milk than with cow's milk, while the volume secreted during the second six hours was practically the same on the two feeds (0.83 per cent greater with soybean milk). Again

TABLE XX

VOLUME OF GASTRIC SECRETION IN PAVLOV POUCHES - TWELVE-HOUR TRIALS

Period	When fed whole cow's milk		When fed soybean milk		Per cent secretion with soybean milk is of that with cow's milk
	Total secretion	Mean half-hourly secretion	Total secretion	Mean half-hourly secretion	
	cc.	cc.	cc.	cc.	
Calf 47U					
12 hours	202.79	8.45	245.08	10.21	120.85
1st 6 hrs.	109.02	9.09	148.73	12.39	136.42
2nd 6 hrs.	93.77	7.81	96.35	8.03	102.75
Calf 47A1					
12 hours	253.07	10.54	294.63	12.28	116.42
1st 6 hrs.	137.30	11.44	174.95	14.58	127.42
2nd 6 hrs.	115.77	9.65	119.68	9.97	103.38
Calf 47Y					
12 hours	213.59	8.90	227.14	9.46	106.34
1st 6 hrs.	118.99	9.92	125.46	10.46	105.47
2nd 6 hrs.	94.60	7.88	101.68	8.47	107.48
Calf 47A2					
12 hours	183.85	7.66	190.96	7.96	103.87
1st 6 hrs.	97.90	8.15	113.77	9.48	116.33
2nd 6 hrs.	86.05	7.17	77.19	6.43	89.70
Average					
12 hours		8.89		9.98	111.87
1st 6 hrs.		9.65		11.73	121.41
2nd 6 hrs.		8.13		8.23	100.83

the peak of secretion, that is, the largest volume of juice secreted in any half-hour period was on the average, 39.35 per cent greater with the soybean diet than with the check ration (table XXI).

The fact that in this series the total secretion for the twelve-hour period was without exception greater with the soybean diet than with cow's milk, indicated that the protein of the soybean is somewhat more effective as a secretagogue than are the proteins of cow's milk. Since the soybean flour in water constitutes a mixture which has many of the characteristics of a "soup", it is interesting to note Howell's (45) statement "meat extracts, meat juices, soups, etc., are particularly effective in this respect (referring to secretagogic effect); milk and water cause less secretion". Furthermore Pavlov (72) found that the total quantity of juice poured out and the duration of its secretion are seen to be dependent upon the kind of food. This relationship is equally clear whether, in estimating the food, one takes its total weight, or its amount of dried substance, or, lastly, its content of nitrogen (since the gastric juice acts only on the protein constituents). While the protein content of the soybean diet was approximately 20 per cent higher than that of the whole milk used for a check, the total secretion on the soybean feed was 11.87 per cent greater than that on milk - a relationship which tends at least to bear out the findings of Pavlov.

Dividing the total volume of secretion by the number of hours through which the trial was conducted gives a figure designated by Pavlov as the "mean hourly rate of secretion". This figure, according to Pavlov, differs for different

TABLE XXI

SHOWING PEAK OF SECRETION (LARGEST VOLUME OF GASTRIC JUICE SECRETED IN ONE HALF-HOUR PERIOD) AND THE PERIOD IN WHICH IT OCCURRED

Calf	When fed cow's milk		When fed soybean milk		Per cent secretion on soybean milk is of that on cow's milk
	Highest reading	Period occurring	Highest reading	Period occurring	
	cc.		cc.		
47U	12.78	1st	19.20	3rd	150.24
47A1	14.49	2nd	24.93	2nd	172.05
47Y	12.83	2nd	12.38	3rd	96.49
47A2	10.75	2nd	14.90	2nd	138.60
Average					139.35

sorts of food. For example he found that on the basis of equivalent weights, flesh requires the most and milk the least gastric juice. In the twelve-hour experiments herein discussed, in which the volume of secretion was determined at half-hour intervals, the mean "half-hourly" rate for milk was 8.89 cubic centimeters while that for soybean milk was 9.98.

As mentioned earlier, practically all of the greater secretion on the soybean diet occurred during the first six hours of the experimental period, while no appreciable difference was found during the last half of the period (table XX). This fact indicates that the soybean diet passed from the stomach more rapidly than the check meal, since for the two diets to have passed from the stomach at equal rates, the curves produced by plotting the respective volumes of gastric secretion would have been parallel. Instead they approached each other as the 12-hour period progressed.

Espe and Cannon (27) found that a test meal of a liter of whole milk containing approximately 3 per cent of butterfat passed from the stomach of a calf in an average of fourteen and one-half hours. Therefore there should be some milk curd in the stomachs of calves at the end of twelve hours, the length of the experimental periods used in this series of trials. Because of the presence of this curd more gastric juice would be secreted than if the stomach were empty so from the rate of gastric secretion a determination of the presence of food in the stomach can be made.

The continued secretion of a volume of gastric juice equal to that produced when cow's milk was fed indicates that some of the soybean material remained in the stomach for at least twelve hours because a liter of milk has not left the stomach by that time. That is to say, if the presence of milk curd in the stomach stimulated the fundic glands of the Pavlov pouch to secrete from 86.05 to 115.77 cubic centimeters of gastric juice in the last six hours of the experimental period (table XX), it is reasonable to assume that some soybean nutrients must have been present in the stomach to produce from 77.19 to 119.68 cubic centimeters in the same period.

Here it should be remembered that the digestion of milk proceeds at a comparatively slow rate, due to the formation of a curd by the action of rennin immediately upon the passage of the milk into the stomach, which curd, being one large mass, permits the gastric juice to contact only a portion - its surface. In the gastric digestion of the soybean milk which forms no curd (other than possibly acid flocculation), the entire mass is exposed to the action of gastric juice from the start and thus more rapid digestion is achieved. Moreover it would seem that more nerve endings in the gastric mucosa would be contacted and stimulated by a liquid like soybean milk than by the hard curd of cow's milk which conforms only partially to the contour of the mucosa lining the stomach, and thus the stimulus for the greater volume of secretion with the soybean diet immediately after feeding could be partially accounted for.

The immediate passage of the soybean material from the stomach is not prevented mechanically, as in the case of milk, but must be dependent upon either a settling of the grannular flour to the greater curvature of the stomach, or by a regulation of the pylorus, or a combination of these factors.

The question of the function and regulation of the pylorus is still an open one. According to Dukes (21), "the pylorus is guarded by a band of circular non-striated muscle known as the pyloric sphincter. The purpose of the sphincter is to prevent the stomach contents from entering the intestine in too large amounts". Alvarez (31), 1933, found that the sphincter is not the only factor concerned in the regulation of the emptying of the stomach. He found that the stomach continues to empty itself in a fairly normal manner after the sphincter has been surgically removed.

One of the early theories as to the regulation of the pyloric sphincter was propounded by Cannon (14) and known as the "acid theory of pylorus control". According to the acid theory, acid on the stomach side opens the pylorus, provided the liquid on the intestinal side is alkaline or neutral, and acid on the intestinal side closes the opening. Later Alvarez reported that anything that stimulates the stomach will cause it to empty faster, while anything that stimulates the upper part of the intestine will delay its emptying. Luckhardt, Phillips and Carlson (58) found a definite relationship between pressure within the stomach and the opening of the pylorus, so that when the pressure was increased as by constricting rings traveling toward the duodenum, the pyloric sphincter relaxed. Gianturco (31) concluded in 1933 that:

1. The pylorus responds to approaching waves of peristalsis much as does the adjacent part of the pars pylorica.
2. Food leaves the stomach when the pylorus and the duodenum are relaxed at the same time.
3. Relaxation of the pylorus alone is not followed by passage of food.

While there is little doubt that small portions of the soybean gruel passed into the duodenum immediately upon being swallowed (31) it is doubtful if any great proportion of the meals left the stomach in this manner. Samples of ingesta drawn through Rehfuss tubes by way of rumen fistulae at least sixteen hours after feeding a test meal of "fortified soybean milk" (soybean flour in water and skimmilk), were found to contain considerable soybean flour. Upon the basis of either the acid theory of Cannon or the findings of Alvarez regarding a relationship of the relaxation of the pylorus to that of the duodenum, the fact that the soybean gruel did not form a distinct curd with rennin did not preclude the possibility of its remaining in the stomach for a time sufficient for peptic activity. The formation of a curd by cow's milk has, without question, the effect of retarding its passage from the stomach. That fact, however, does not prove that were it not to form a coagulum, it could leave the stomach immediately. Indeed Mortenson (66) found that a sample of autoclaved milk did not form a curd in the stomach but appeared as a "thick soup" after two hours. Moreover it is common knowledge that human milk forms a curd so soft as to be hardly measurable and does not collect as a solid mass in the infant stomach, yet is retained for peptic digestion.

Anatomical and physiological differences between the gastric system of the human infant and that of the calf may be influential in enabling the infant to retain materials in the stomach which under similar conditions in the calf's stomach would not be held. It is reasonable to suppose that the infant is by nature fitted to handle a more flocculent and less rubbery curd than is the calf. The special value of soft curd milk in infant feeding appears to demonstrate rather conclusively that the infant stomach is adapted to a softer curd than that formed by average cow's milk. Again the Chinese workers (94, 96, 93, 16, 95, 87) have been peculiarily successful in feeding soybean gruel to infants, and even American investigators and pediatricians (78, 79, 88, 40, 41, 77, 92, 52) have obtained good results with infants who could not tolerate cow's milk, the only comment being that while the stools were usually found to be somewhat more loose than with milk, the looseness never reached a diarrhea.

Sisson (89), reasoning from the fact that the pylorus of an embalmed horse is somewhat relaxed, considered the pylorus in this animal to remain open normally. On the other hand, Dukes (21) stated that at least in the carnivores and man the pylorus is normally closed. The fact that digestion is a continuous process in ruminants so that generally speaking only liquid or semiliquid ingesta reaches the true stomach, renders it a more or less reasonable assumption that the regulation of the pylorus is of less importance in this class of animals than in single stomached animals. It does not prove however that the pylorus has no function in the passage of food from the ruminant stomach. The fact that soybean flour was found in samples of stomach ingesta removed after sixteen hours does prove that such material may be held in the stomach without the formation of a definite curd.

Continuous Trials

The results of the series of continuous trials with calves full-fed three times each day, are shown in figures 14, 15, 16 and 17. In most cases the curves plotted represent averages of seven days on a diet of cow's milk and an equal time on simple soybean milk. However, calves 47Y and 47A11 were not used during the entire fourteen days (table X). As would be expected the volume of secretion of gastric juice, with few exceptions, showed a characteristic increase following each feeding. Since the feed was given at intervals of eight hours, the abomasum of the calf was never empty, at least upon the check meal of whole cow's milk, for as stated earlier, it was found that a liter of milk containing 3 per cent of fat remains in the abomasum an average of approximately fourteen and one-half hours. In these trials the meal consisted of four pounds in the case of calves 47Y and 47A2, and of three pounds in the case of calves 47A11 and 47A12. Therefore it is clear that one meal had not completely left the stomach before the next was introduced. For this reason the minimum secretion did not reach a point as low as with the single trials, preceded by a fasting period. With calves 47Y and 47A2 which were used in both twelve-hour and continuous trials, the point of minimum

Form E-3

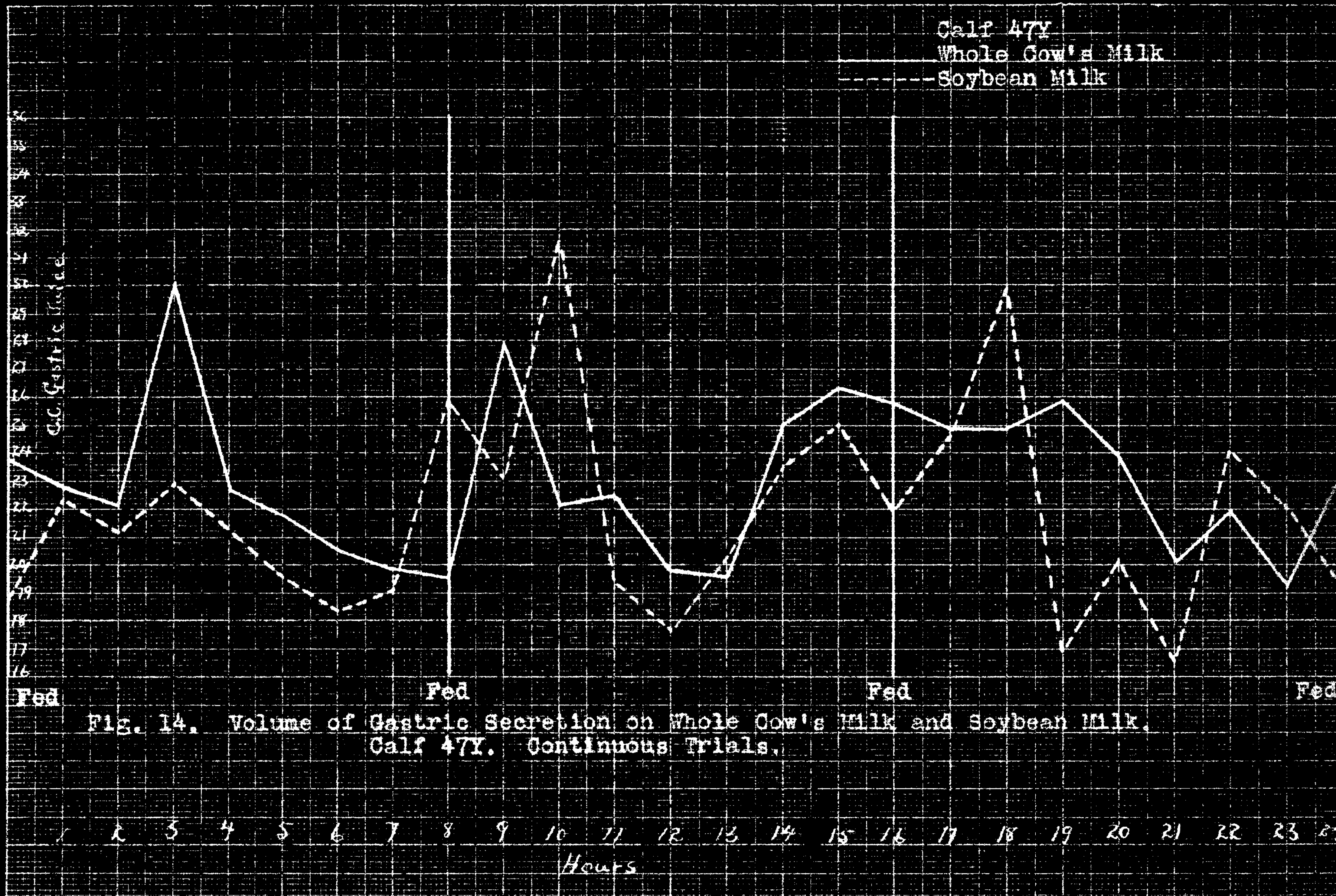


Fig. 14. Volume of Gastric Secretion on Whole Cow's Milk and Soybean Milk. Calf 47Y. Continuous Trials.

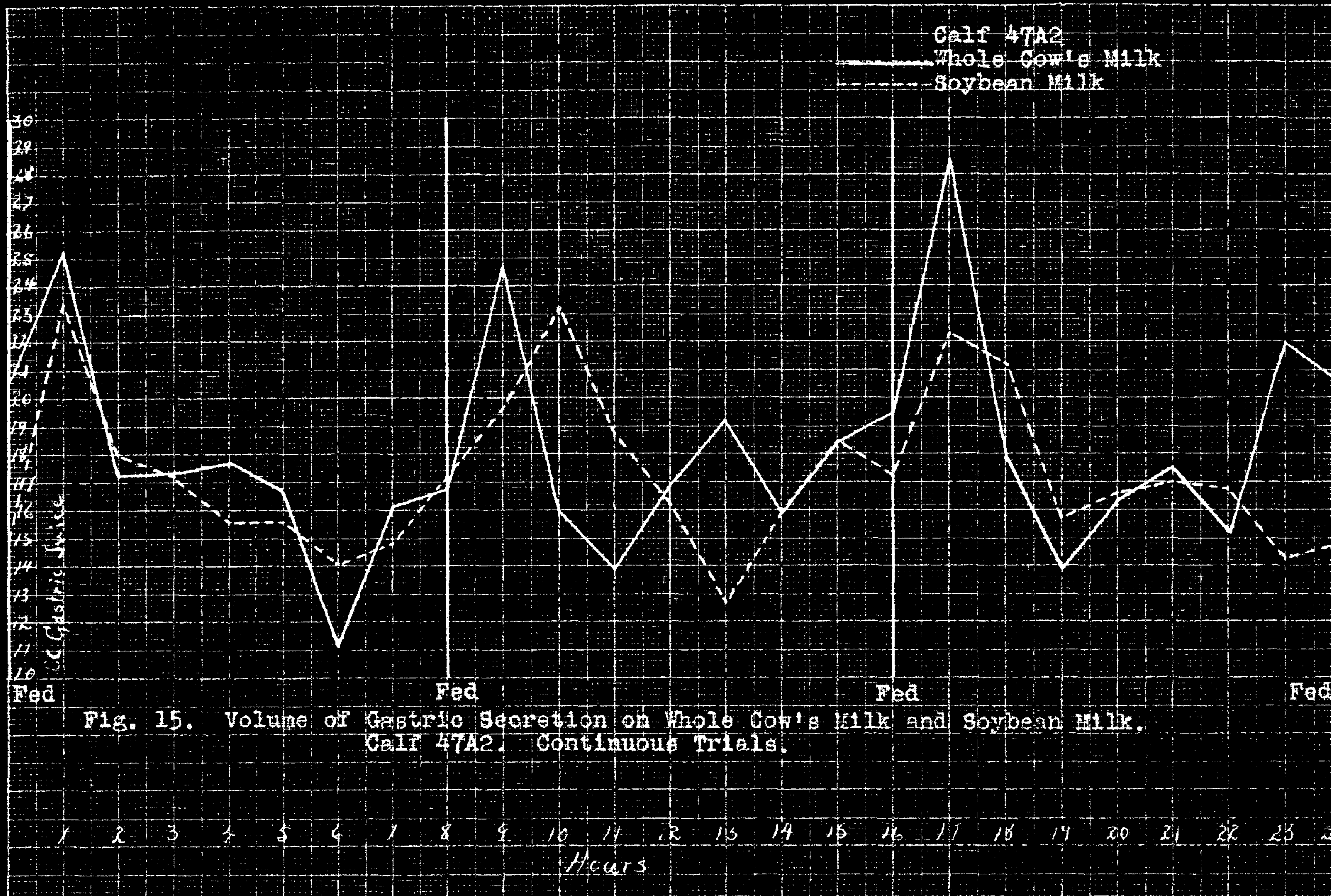


Fig. 15. Volume of Gastric Secretion on Whole Cow's Milk and Soybean Milk. Calf 47A2. Continuous Trials.

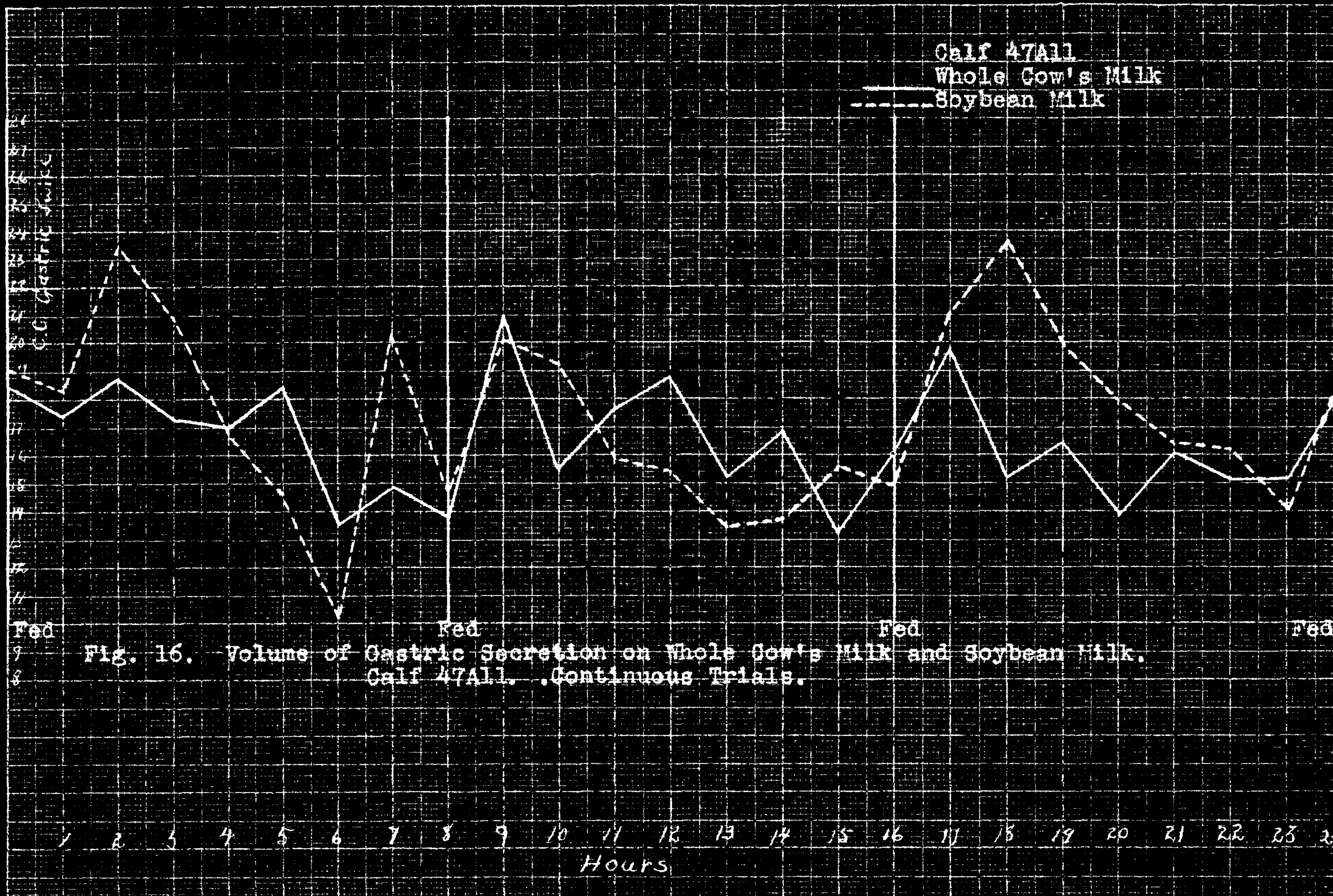


Fig. 16. Volume of Gastric Secretion on Whole Cow's Milk and Soybean Milk.
Calf 47A11. .Continuous Trials.

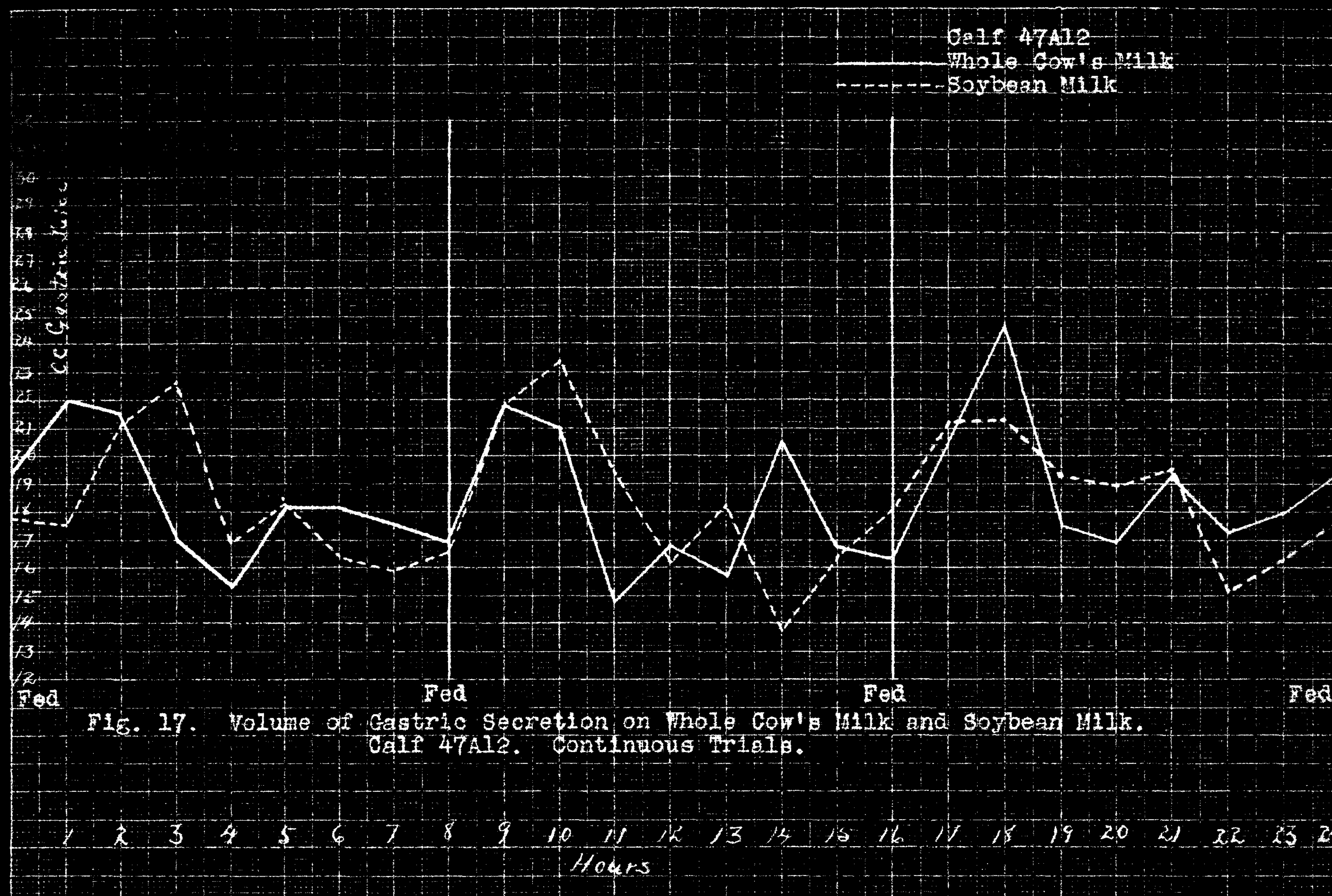


Fig. 17. Volume of Gastric Secretion on Whole Cow's Milk and Soybean Milk.
Calf 47A12. Continuous Trials.

secretion was higher in all cases in the continuous than in the twelve-hour experiments. A comparison of the maximum and minimum secretion in these calves is made in table XXII. It may be seen from this table that the points of maximum secretion were higher, with one exception, in the continuous than in the twelve-hour trials. Calf 47A2 showed virtually the same point of maximum secretion in both series. This higher maximum would be expected since the amount of nutriment in the stomach was thought to be greater throughout the experimental day. It does not differ in proportion to the difference in amounts fed however. That is, according to Pavlov, a meal of four pounds of milk should excite almost twice the secretion produced by one liter (approximately 2.3 pounds). It should be recalled however that Pavlov's observations were made upon a single stomached animal (dog), while in the calf the digestion mechanism is designed for a continuous process of digestion. Moreover, had Pavlov increased the amount of his test meals to the point where they would have represented full meals for the animals so that the effect of hunger would have been eliminated, probably the ratios he established would not have been maintained on the heavier feeding. The use of a fasting period followed by a small test meal sets up an artificial condition for digestive activity which may cause a distorted effect. Even though this may be true it does offer the optimum opportunity for secretagogic effect. It is quite

TABLE XXII

COMPARISON OF MAXIMUM AND MINIMUM HALF-HOURLY SECRETION OF CALVES 47Y AND 47A2 IN TWELVE-HOUR AND CONTINUOUS EXPERIMENTS. (CUBIC CENTIMETERS)

	Feed used	Twelve-hour series	Continuous series	Per cent continuous is of 12-hour secretion
		Calf 47Y		
Minimum secretion	Whole milk	6.58	8.72	132.52
Maximum secretion	Whole milk	12.83	16.92	131.88
Minimum secretion	Soybean milk	7.22	7.32	101.39
Maximum secretion	Soybean milk	12.38	16.07	129.81
		Calf 47A2		
Minimum secretion	Whole milk	4.90	5.16	105.31
Maximum secretion	Whole milk	10.75	15.19	141.30
Minimum secretion	Soybean milk	4.03	5.90	146.40
Maximum secretion	Soybean milk	14.90	14.63	98.19

possible that a fasting period gives opportunity for the "storage", so to speak, of humoral material, gastrin, which is able to exert its influence upon gastric secretion whenever food again appears in the stomach. Such a condition would partially explain the fact that in the continuous trials the maximum half-hourly secretion was only twenty to thirty per cent greater after the meal of four pounds than upon the meal of one liter or 2.3 pounds.

Further explanation for the failure of the maximum secretion to rise in proportion to the amount of the meal is found in the fact that not all of the meal was in the abomasum at any one time. It was observed in the fistula calves that immediately after a full meal was fed a considerable part of the meal had passed into the rumen and was therefore not in the true stomach. An electric light held inside the rumen while such calves were drinking revealed that the first portion of the meal passed directly into the abomasum, no doubt satisfying immediate hunger, the esophageal groove being held closed to the rumen outlet. Often the first swallow or two would pass into the rumen, indicating a relaxed condition of the groove, after which it would close tightly and permit no more to pass over the lips. As the calf continued to drink however, the groove again relaxed, allowing the remainder of the meal to pass into the rumen. The palatability of the meal also appeared to influence the rate of its passage into the rumen. Upon a meal of milk the groove seemed to remain closed longer, thus allowing a greater portion of the meal to pass directly into the abomasum, than with a meal of the soybean gruel, which was less palatable. In other words in the ruminant there seems to be a means of controlling the amount of food in the abomasum which is not found in single-stomached animals, that is, by regulating the passage of food either into the rumen or into the abomasum where actual enzymatic digestion occurs. Also when the full meals were fed fistula calves, by tubes, in which case they were placed in the true stomach, often a part of a meal would soon return to the rumen so that it seems the passage of food material in either direction past the reticulo-omasal orifice, occurs as a means of regulating the quantity of material in the true stomach. The control of this activity may be a matter of pressure adjustments in the reticulum and abomasum, but is probably reflex, since the movements of the stomach are, in general, automatic (21). A definite muscular constriction is felt upon passing the fingers into the orifice. If the reticulo-omasal orifice has no positive function in the regulation of the passage of ingesta from the rumen into the abomasum and the flow of material through this orifice is only a matter of pressure adjustment, such regulation would hinge considerably upon the regulation of the pylorus.

The results obtained from continuous trials using soybean milk and whole cow's milk, are summarized in table XXIII. From this table it will be seen that in most cases the total secretion when the calves were fed soybean gruel was slightly less than that on cow's milk, although calf 47A11 produced a larger total secretion on soybean milk than on cow's milk. That most of the calves should secrete more gastric juice when fed cow's milk than when fed soybean milk appears in direct conflict with the results obtained in the twelve-hour trials in which, it will be recalled, the secretion on soybean milk was constantly greater than that on cow's milk. The total gastric secretion in the continuous trials averaged

TABLE XXIII

VOLUME OF GASTRIC SECRETION IN PAVLOV POUCHES IN CALVES FED WHOLE COW'S MILK OR SOYBEAN MILK EVERY EIGHT HOURS

Period	When fed whole cow's milk: Total secretion	When fed whole cow's milk: Mean half-hourly secretion	When fed soybean milk: Total secretion	When fed soybean milk: Mean half-hourly secretion	Per cent secretion on soybean milk is of that on whole milk
	cc.	cc.	cc.	cc.	
Calf 47X					
24 hours	552.54	11.51	515.19	10.73	93.24
1st 8 hrs.	178.90	11.18	170.52	10.66	95.35
2nd 8 hrs.	189.04	11.82	182.74	11.42	96.62
3rd 8 hrs.	184.60	11.54	161.93	10.12	87.69
Calf 47A2					
24 hours	434.67	9.06	418.07	8.71	96.18
1st 8 hrs.	137.93	8.62	136.34	8.52	98.84
2nd 8 hrs.	144.75	9.05	142.72	8.92	98.56
3rd 8 hrs.	151.99	9.50	139.01	8.69	91.47
Calf 47A11					
24 hours	396.39	8.26	416.57	8.68	105.09
1st 8 hrs.	131.25	8.20	139.38	8.71	106.22
2nd 8 hrs.	134.72	8.42	128.65	8.04	95.49
3rd 8 hrs.	130.42	8.15	148.54	9.28	113.87
Calf 47A12					
24 hours	443.81	9.25	442.6	9.22	99.73
1st 8 hrs.	146.51	9.16	145.47	9.09	99.24
2nd 8 hrs.	143.78	8.99	147.28	9.21	102.45
3rd 8 hrs.	153.52	9.60	149.86	9.37	97.60
Average					
24 hours		9.52		9.34	98.56
1st 8 hrs.		9.29		9.25	99.91
2nd 8 hrs.		9.57		9.40	98.28
3rd 8 hrs.		9.70		9.37	97.66

approximately two per cent greater with milk feeding than with soybean milk, while in the twelve-hour series (table XX) the difference was near twelve per cent greater when soybean milk was fed than with cow's milk.

An entirely satisfactory explanation for this more or less reversal of results cannot be offered at present. It might be suggested that the remainder of portions of a test meal in the rumen for a time changes or inactivates its capacity as a secretagogue, or that the secretagogic capacity is arrested by the presence of food in the stomach. This condition then would explain the relatively smaller volume of juice on the soybean gruel in these trials. The 11.87 per cent greater secretion on soybean milk in the twelve-hour experiments is attributed largely to the secretagogic capacity of the soybean material.

The loss of secretagogic capacity might reasonably explain the fact that the maximum half-hourly volumes of secretion in the continuous trials did not increase in proportion to the amount of feed given, but it does not explain why the total secretion on twelve pounds (feed for one 24-hour period) did not excite the secretion of a volume of juice five times that secreted by the same calf when fed one liter (approximately one-fifth the amount of feed). If, as Pavlov reported, the volume of secretion is in direct ratio to the amount of feed acted upon, it would be expected that a calf would secrete five times the volume of juice in digesting twelve pounds of feed that it would produce in the digestion of one-fifth that amount. Actually the volume of secretion on the one liter meal was from 38.66 to 45.68 per cent of that on the twelve-pound feeding, in calves 47Y and 47A2, as is seen from table XXIV below:

TABLE XXIV

COMPARISON OF GASTRIC SECRETION ON ONE LITER MEAL WITH A MEAL OF TWELVE POUNDS FED TO CALVES 47Y AND 47A2

Diet	1 liter Twelve-hour trials cc.	12 lbs. Continuous trials cc.	Per cent twelve-hour is of continuous secretion
	Calf 47Y		
Cow's milk	213.59	552.54	38.66
Soybean milk	227.14	515.19	44.09
	Calf 47A2		
Cow's milk	183.65	434.67	42.30
Soybean milk	190.96	418.07	45.68
	Average		
Cow's milk			40.48
Soybean milk			44.89

Here again the findings of Pavlov may not be applicable in that he did not increase the test meals to the full digestive capacity of his animals, so that the ratio of secretion to the amount of food, established in hungry animals probably would not have held if the animals had been full-fed.

The results roughly compared in table XXIV were obtained under very different conditions. First, in the twelve-hour trials the calves were fasted previous to being fed the test meal, which assured that the stomach was empty of previous feedings. On the other hand in the continuous feedings no attempt was made to empty the stomach between feedings. Under the conditions which prevailed in the twelve-hour trials, opportunity was given for the maximum effect of secretion of gastric juice in the immediate satisfaction of hunger. Feeding after a fast resulted in the passage of all the meal directly into the abomasum where it had opportunity to stimulate the nerve endings in the gastric mucosa without first being diluted by rumen contents.

In the continuous trials some of each meal was probably exposed for some hours to the effects of rumen activity, for it was observed with calves 47Y and 47A2, which had gastric fistulae, that when large test meals were placed directly in the abomasum by means of a rubber tube, it was only a few minutes after feeding that a portion of the meal would be found in the rumen even though the rumen was empty when the meal was given. This rumen activity of a portion of the meal may have altered its status as a secretagogue and thus changed its effect on gastric secretion, upon reaching the true stomach.

Although the existence of a psychic phase of gastric secretion in ruminants is doubted (20), there is some indication in the results of this series of continuous trials that such may exist. While the secretion was expected to fall gradually just previous to each feeding, in half the cases the volume of juice, as may be seen in figures 14, 15, 16,and 17, increased just previous to the feeding of the next meal. It is possible that this increase was due merely to greater physical activity on the part of the calves, thus effecting a more nearly complete emptying of the pouch. Psychic secretion, if it exists, would be affected by the number of feedings given, the extent of hunger, etc.

Series With Fortified Soybean Milk

As mentioned earlier the sixteen-hour experiments involving fortified soybean milk (soybean flour and water, with the addition of skimmilk and calcium chloride), were conducted in two divisions. These were, first, a series of experiments to determine the volume of gastric secretion by use of calves with Pavlov pouches, and second a series to determine the acidity of gastric contents by use of rumen fistulae, through which samples of abomasal contents were drawn periodically.

Volume of Gastric Secretion

The results of the series of experiments using five calves with Pavlov pouches to determine gastric digestion of fortified soybean milk by measuring the volume of gastric juice secreted at half-hour intervals, are shown graphically in figures 18, 19, 20, 21, and 22. The volumes and means

Fig. 18. Volume of Gastric Secretion on Skimmilk and Fortified Soybean Milk.
Calf 1344. Sixteen-Hour Trials

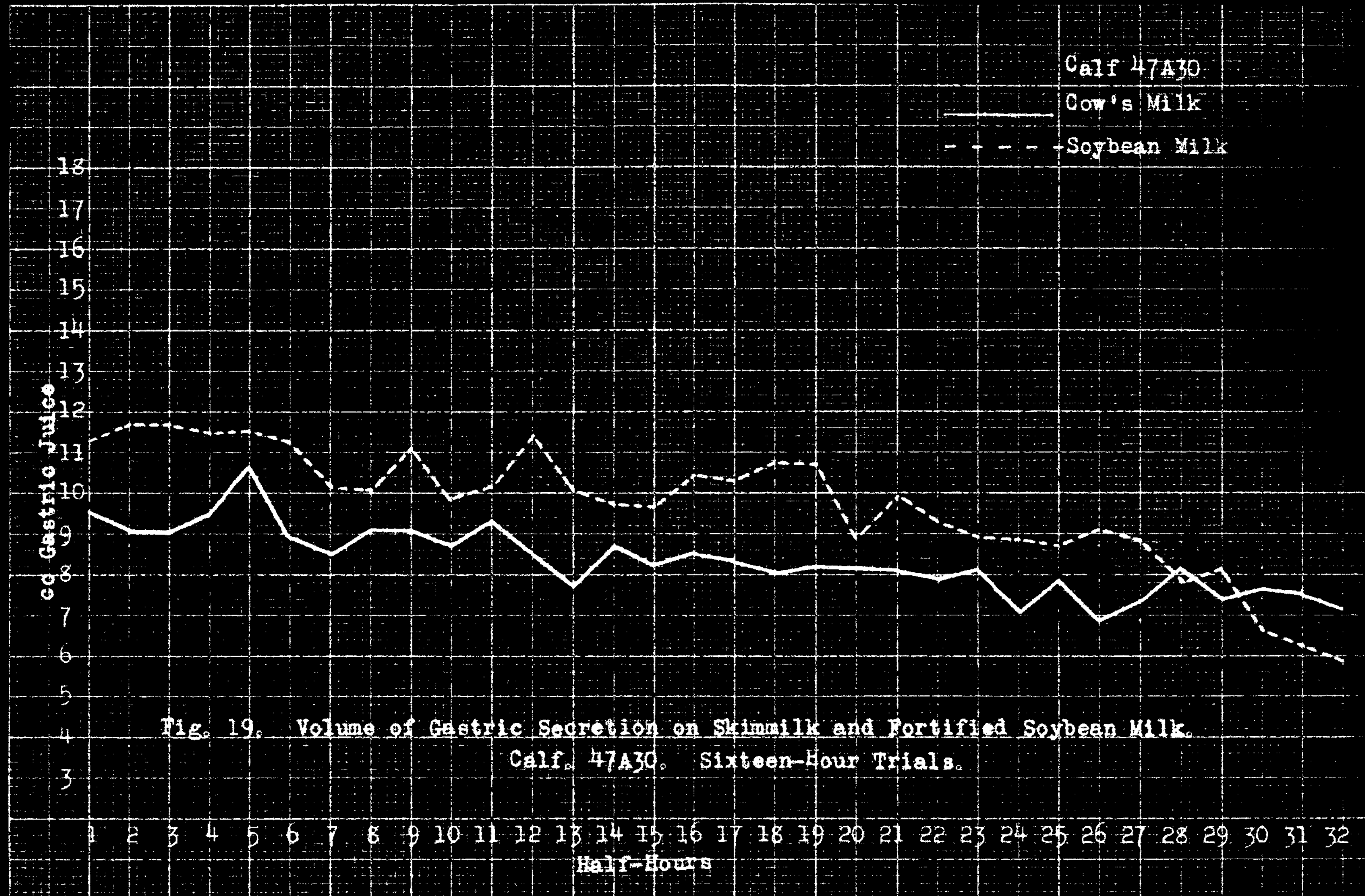


Fig. 19. Volume of Gastric Secretion on Skimmilk and Fortified Soybean Milk.
Calf. 47A30. Sixteen-Hour Trials.

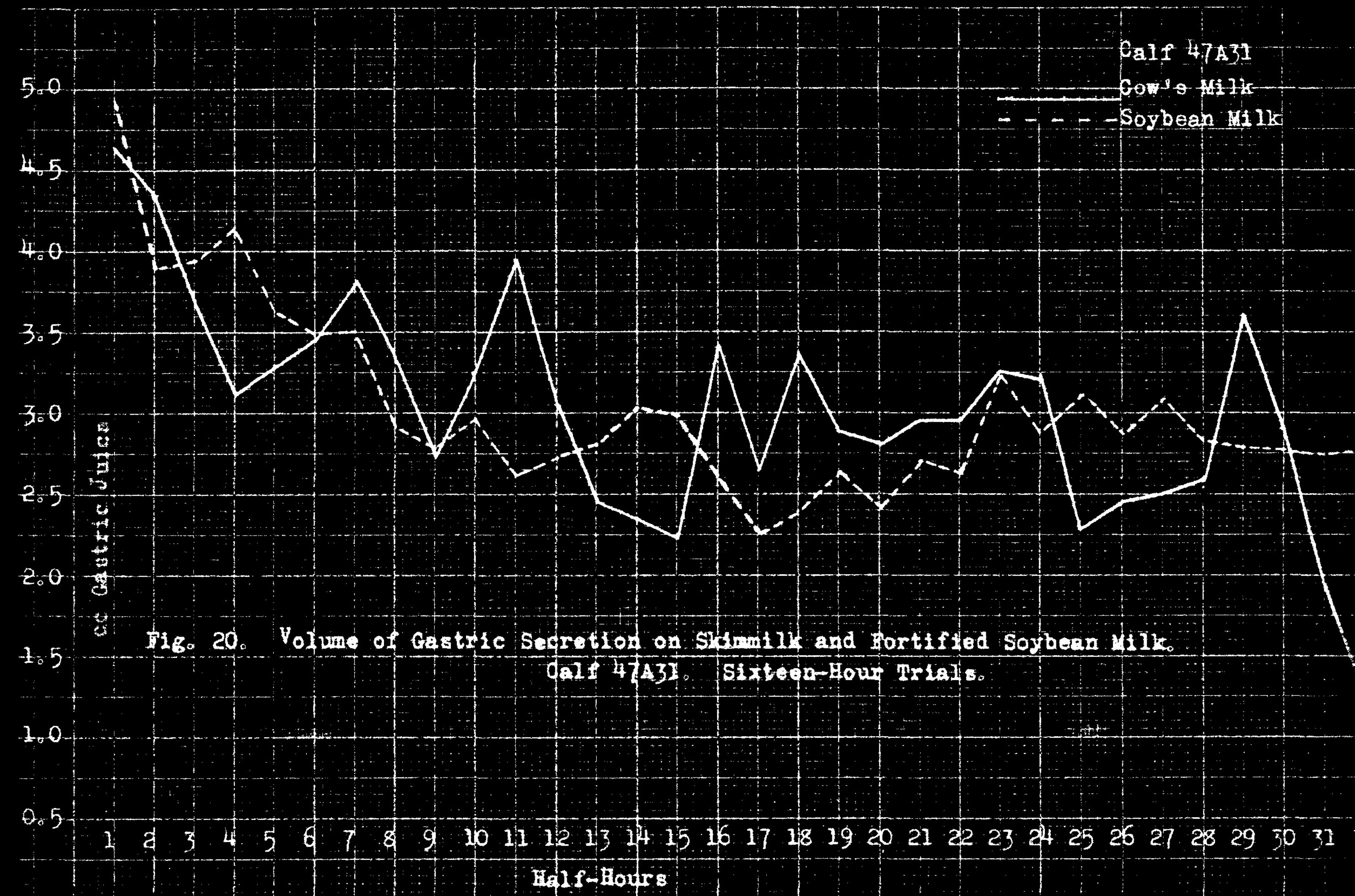


Fig. 20. Volume of Gastric Secretion on Skimmilk and Fortified Soybean Milk.
Calf 47A31. Sixteen-Hour Trials.

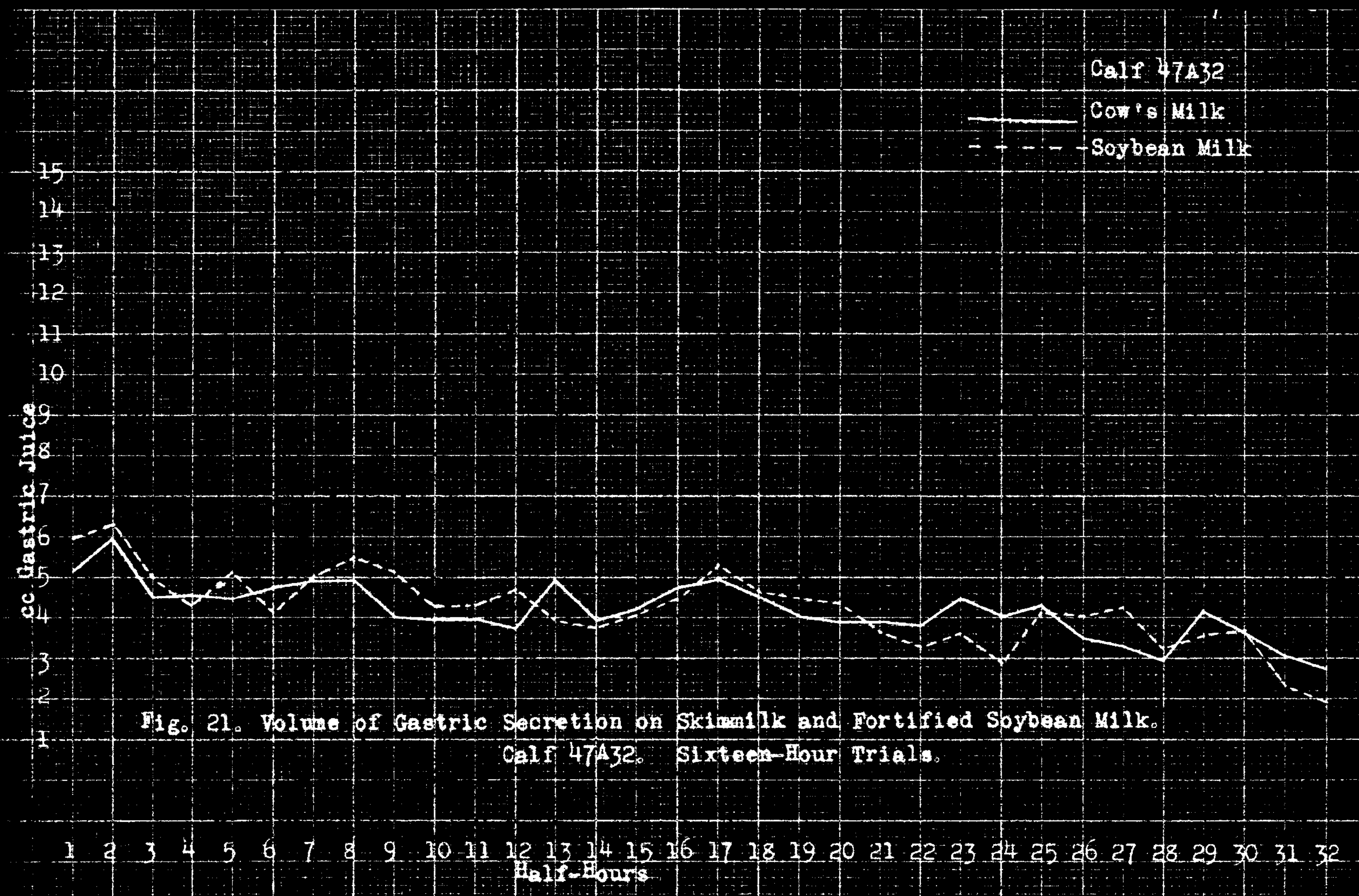


Fig. 21. Volume of Gastric Secretion on Skimmilk and Fortified Soybean Milk. Calf 47A32. Sixteen-Hour Trials.

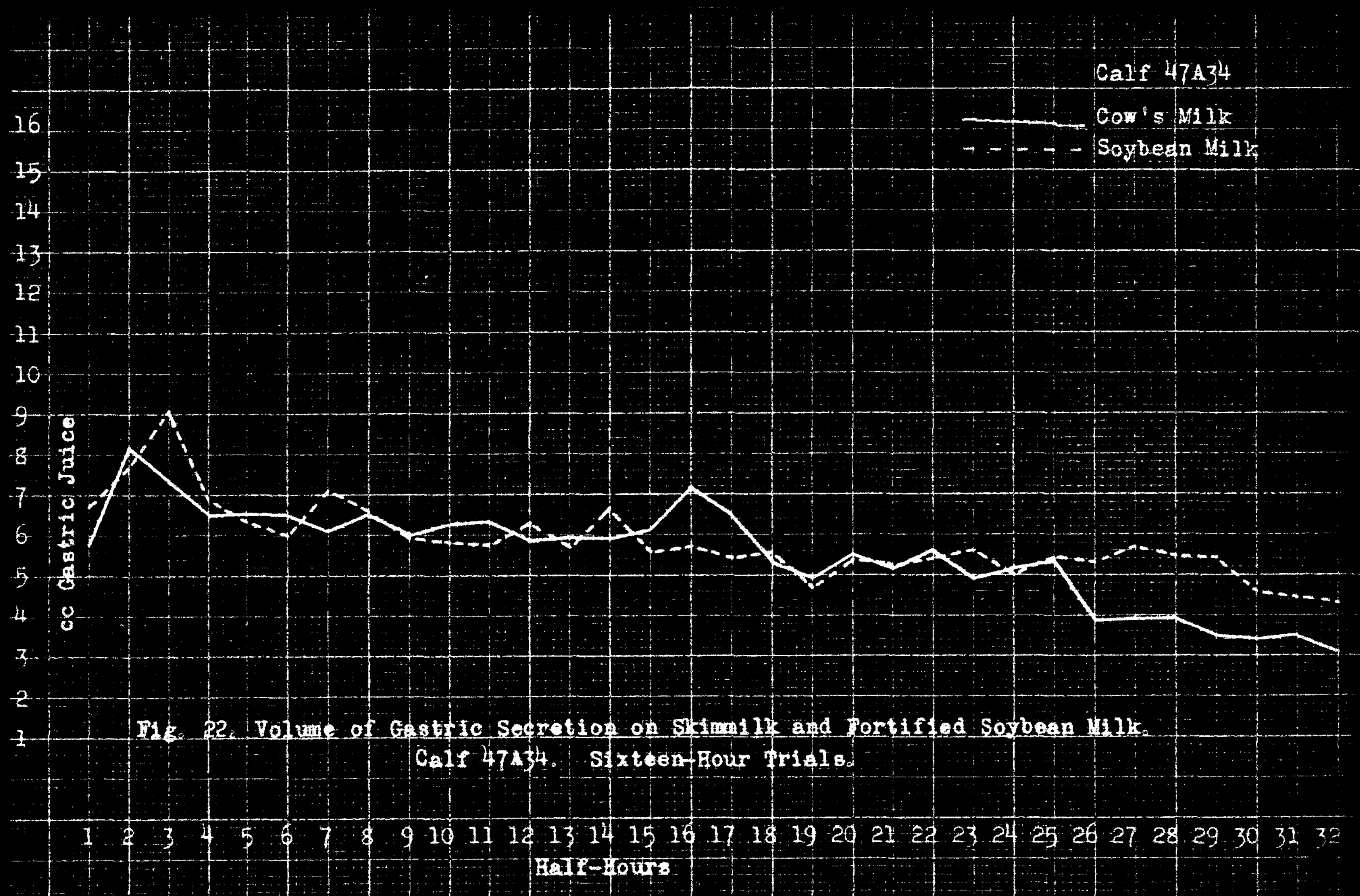


Fig. 22. Volume of Gastric Secretion on Skimmilk and Fortified Soybean Milk.
Calf 47A34. Sixteen-Hour Trials.

of secretion, together with the per cent that the secretion on fortified soybean milk is of that on skimmilk, are given for the individual calves in table XXV.

From these data it will be seen that the results of the sixteen-hour series followed rather closely those from the twelve-hour series, discussed earlier. The total volume of secretion produced by the soybean diet was without exception greater than that produced by skimmilk, which fact is in accord with the results of the twelve-hour experiments. The difference in volume, however, was considerably less in the sixteen-hour series, so that while an 11.87 per cent greater volume of juice was produced by the soybean gruel in the twelve-hour series (table XX), a difference of only 5.8 per cent was produced by the fortified soybean milk in the sixteen-hour experiments (table XXV).

Again in the sixteen-hour series, the soybean diet showed a less marked tendency to produce a heavy secretion during the early hours of the experimental period. A comparison of the first eight hours of the experimental period with the second eight hours, reveals that the secretion on fortified soybean milk was only 7.71 per cent greater during the first half of the experimental period, then that on the skimmilk, and that it remained greater by 3.28 per cent through the second eight-hour period. It will be recalled that in the twelve-hour experiments the secretion on simple soybean milk (soybean flour

TABLE XXV

SHOWING VOLUMES, AND MEANS OF GASTRIC SECRETION ON SKIMMILK AND FORTIFIED SOYBEAN MILK IN SIXTEEN-HOUR EXPERIMENTS WITH PAVLOV POUCH CALVES

Period	When fed skimmilk		When fed fortified soybean milk		Per cent secretion on soybean milk is of that on skimmilk
	Total secretion	Mean half-hourly secretion	Total secretion	Mean half-yourly secretion	
	cc.	cc.	cc.	cc.	
Calf 1344					
16 hours	280.76	8.77	295.78	9.24	105.35
1st 8 hrs	167.62	10.48	189.94	11.87	113.25
2nd 8 hrs	113.04	7.06	105.84	6.62	93.63
Calf 47A30					
16 hours	266.94	8.34	310.60	9.71	116.36
1st 8 hrs	142.92	8.93	171.50	10.72	120.00
2nd 8 hrs	124.02	7.75	139.10	8.69	112.16
Calf 47A31					
16 hours	96.32	3.01	96.76	3.02	105.64
1st 8 hrs	52.80	3.30	52.70	3.29	100.84
2nd 8 hrs	43.52	2.72	44.06	2.75	112.33
Calf 47A32					
16 hours	133.77	4.18	135.36	4.23	101.19
1st 8 hrs	72.61	4.54	76.00	4.75	104.67
2nd 8 hrs	61.16	3.82	59.36	3.71	97.06
Calf 47A34					
16 hours	176.74	5.52	186.70	5.83	100.46
1st 8 hrs	102.92	6.43	103.78	6.49	99.81
2nd 8 hrs	73.82	4.61	82.92	5.18	101.24
Average					
16 hours					105.80
1st 8 hrs					107.71
2nd 8 hrs					103.28

in water) was 21.41 per cent higher than the secretion on whole cow's milk, during the first half of the twelve-hour period, but that the secretions were virtually equal during the second half. That is, the fluctuation on either side of the mean difference in volume of secretion was much less in the sixteen-hour series with fortified soybean milk, than in the twelve-hour experiments in which simple soybean flour in water, was used. In the case of calf A31, the secretion on fortified soybean milk was even slightly less during the first eight hours, than that on skimmilk. The spread between the maximum and minimum difference in volume of secretion on the cow's milk and soybean milk, for the first and second halves of the observation periods, respectively, reached 20.58 per cent in the twelve-hour trials, but amounted to only 4.43 per cent in the sixteen hour experiments.

In general it is seen that the curves (figures 18-22) of volume of secretion recorded from the sixteen-hour trials with fortified soybean milk are more nearly like those recorded from skimmilk in the same series, than was true of the soybean milk and whole cow's milk in the twelve-hour trials (figures 10-11-12 and 13). This greater similarity in volume of secretion between fortified soybean milk and skimmed milk is interpreted to mean that in the calf's stomach, the fortified soybean milk was rather more similar to cow's milk in its ability to stimulate the flow of gastric juice than the simple mixture of soybeans and water.

The cause or causes for the difference in results noted between the twelve-hour series and the sixteen-hour trials, must be sought in the difference between the rations used. There was however a difference of four hours in the length of the experimental day. Since the twelve-hour series was run, it has been found that a liter of whole milk containing 3 per cent of fat, disappears from the stomach of the calf in approximately fourteen hours (27), so that the stomach had not completed the work of digesting the test meals in the twelve-hour trials, by the end of the experimental day. This difference could not account for the great height to which the secretion on soybean milk rose, however, during the first few hours of the twelve-hour experiments, which extreme rise did not occur in the sixteen-hour series.

Four important differences in the rations used in the two series of experiments under consideration, were without doubt instrumental in producing the differences in the results obtained. First, the soybean diet used in the sixteen-hour trials and referred to as "fortified soybean milk", contained skimmed cow's milk. Approximately a third of the total nutrients of this gruel consisted of skimmed milk. This was expected to have the effect of rendering the secretion on the soybean diet nearer that on cow's milk. The addition of this amount of cow's milk changed the nutritive ratio of the soybean diet from 1:1.1 for soybean flour in water, as used in the twelve-hour experiments, to 1:1.3 for the fortified soybean milk, used in the sixteen-hour series, since the protein content of the skimmilk is lower than that of soybean flour. Inasmuch as the protein is the nutrient responsible for the excitation of gastric juice, according to Pavlov (72), it is entirely reasonable to suspect that the presence of cow's milk in the fortified soybean gruel would tend to lessen the gastric juice which would be produced by the fortified soybean milk.

The second difference in the rations of the sixteen-hour series is the presence of calcium chloride, which was added to the fortified soybean milk, to strengthen the curd formed by the cow's milk which the mixture contained.

In spite of the fact that, measured by the Hill test, the fortified soybean milk had a curd tension of 50 grams or more, the samples withdrawn from the calves' stomachs were merely a very thick soup, or almost a dough, but with less tenacity, and did not resemble closely the in vitro samples used in the Hill test. The difference was probably due to further dilution with water in the stomach, which was sufficient to practically break the curd.

The fact that in the last half of the experimental period in the sixteen-hour trials the volume of juice secreted on the fortified soybean milk diet was 3.28 per cent higher than that on skimmed milk, whereas in the corresponding half of the twelve-hour trials, the secretion on simple soybean milk was approximately equal to that on whole cow's milk, indicates that at least a greater percentage of the soybean test meal remained in the stomach during this time as a cause for peptic activity in the case of the fortified soybean milk than occurred in the twelve-hour experiments.

It does not seem that the presence of this calcium salt in the stomach should have, of itself, an exciting influence upon the gastric glands. Pavlov investigated a number of inorganic substances as excitors of gastric secretion, including the constituents of meat-ash, which no doubt contained calcium salts, chloride and bicarbonate of sodium, and hydrochloric acid, and found that of the group, only sodium bicarbonate had any influence on the secretory activity of the stomach, and this influence was inhibitory. It is possible that the characteristic of the soybean flour which produced the secretagogic effect to which much of the extreme rise in secretion on this diet in the twelve-hour trials was attributed, was either destroyed or masked by the calcium salt, so that this effect was not seen in the sixteen-hour trials.

The third difference between the soybean diet used in the twelve-hour trials and the fortified soybean milk, was that in the latter the flour was mixed with skimmilk and water in such proportions as to produce a gruel containing 20 per cent dry matter. Thus the fortified soybean milk containing twice the amount of dry matter that the skimmilk contained, was fed in half the volume - the test meal being a half-liter instead of a liter as were the test meals of cow's milk and simple soybean milk.

Pavlov found that water alone has some capacity for exciting the flow of gastric juice. Since the fortified soybean milk contained less water than the simple flour and water mixture, it would be expected to excite somewhat less gastric secretion.

The fourth difference in the rations pertains, not to the soybean diet at all, but to the check meal of cow's milk. In the twelve-hour series, whole milk, testing approximately 3 per cent fat, was used as a check diet, while in the sixteen-hour experiments, skimmilk with a fat content of 0.08 per cent was used.

The effect of fat upon the digestion of other nutrients has been considered by various workers including Pavlov, who found that without exception, less gastric juice was secreted in the presence of fat than in its absence. In dogs with Pavlov pouches and gastric fistulae, he secured a secretion on whole milk almost double that obtained on cream. He reported further that a similar difference occurred when skimmilk was used and compared with whole milk. Upon the basis of the work of Pavlov and others, the opinion seems to be held generally by physiologists that fats retard the rate of digestion. Pavlov offered as an explanation for his results, the theory that fat produces a "reflex inhibition of the secretory process", the reflex originating either in the stomach or upper part of the small intestine.

If fat acts as an inhibitor of gastric secretion, the protein material of the whole milk in the twelve-hour trials was not permitted to exert its full influence upon the gastric glands. That is, a part at least of the difference in the amount of gastric juice produced on simple soybean milk and that produced on whole cow's milk was due to an inhibitory effect of the fat in the whole cow's milk. This would mean then that the difference between volume of secretion on fortified soybean milk and on skimmilk was less in the sixteen-hour series than in the twelve-hour experiments, in part, because the skimmilk, being practically free of fat, excited a greater production of gastric juice than the whole cow's milk.

Recent work by Espe and Cannon (27) does not agree with the generally accepted opinion that fat inhibits digestion in the stomach. These investigators, working with calves, found that skimmilk remained for a longer time in the stomach than did whole milk, and that milk to which cream had been added, making a fat content of 6 per cent, remained in the stomach for an even shorter time than did whole milk. If the time of evacuation of food from the stomach can be taken as an index of the rate of digestion, then this work would appear to be in conflict with that of the earlier workers. In the work of Espe and

Cannon, no measurements of gastric secretion were made, the technique of palpation being used exclusively as a means of determining evacuation time.

Acidity of Gastric Contents

The results of the gastric acidity determinations on skimmilk as compared to fortified soybean milk are given in tables XXVI and XXVII, and in figures 23, 24, and 25. It is seen from these figures and from table XXVI, that the fortified soybean milk diet produced a somewhat higher acid condition in the stomach than did skimmilk. The points of highest total acidity in the three calves ranged from 4.10 to 20.18 per cent higher with the soybean ration than with skimmilk, the average difference being 12.58 per cent. On the other hand the free acid was considerably higher with the skimmilk than with the soybean ration, ranging from 13.33 to 29.31 per cent higher with the skimmilk diet. The average difference was 19.20 per cent for the point of highest free acidity, while the mean difference was 15 per cent higher for the skimmilk ration. Free acidity was determined by use of Topfer's reagent as an indicator, which gives a rather indefinite end-point, so that too much significance cannot be attached to these determinations. However, the differences are sufficiently great to warrant notice, and the determinations on the two rations are at least comparable.

Fig. 23. Free and Total Acid produced on Skimmilk and Fortified Soybean Milk. Calf 1306.

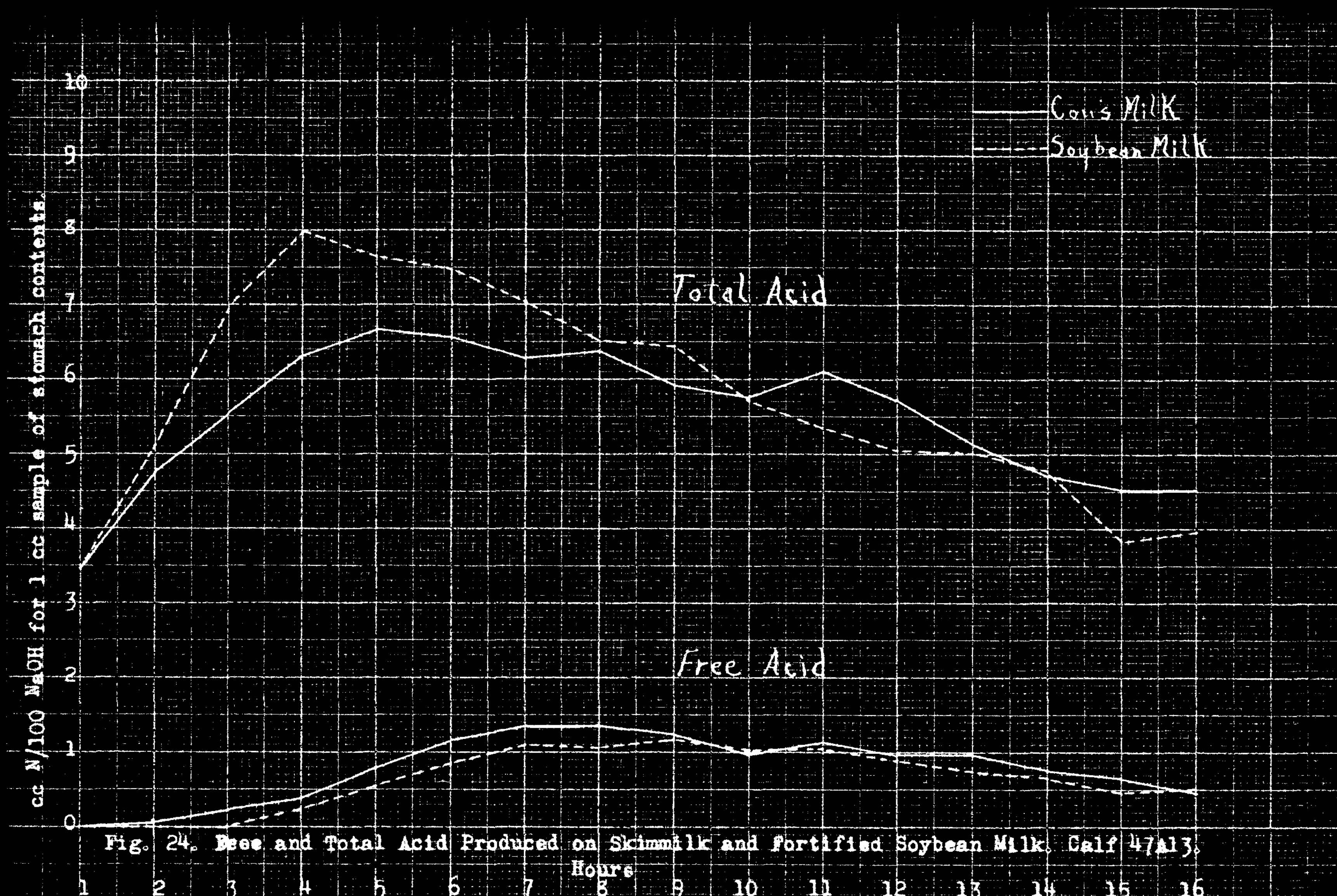


Fig. 24. Free and Total Acid Produced on Skimmilk and Fortified Soybean Milk. Calf 47A13.

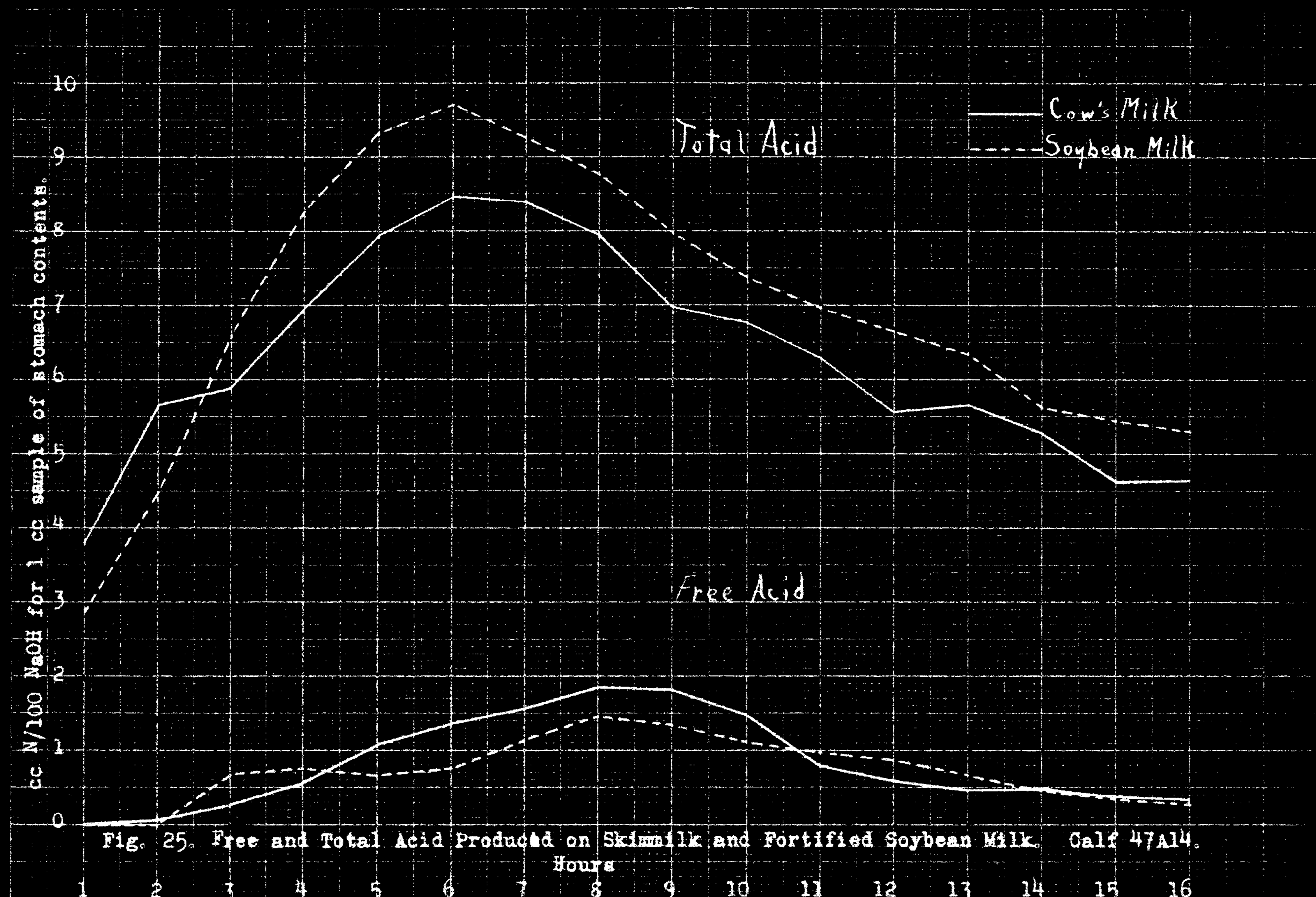


Fig. 25. Free and Total Acid Produced on Skimmilk and Fortified Soybean Milk. Calf 47A14.

TABLE XXVI

SUMMARY OF GASTRIC ACIDITY DETERMINATIONS WITH FISTULA CALVES FED SKIMMILK AND FORTIFIED SOYBEAN MILK

Calf	Free Acid					
	When fed skimmilk			When fed soybean milk		
	Maximum	Minimum	Mean	Maximum	Minimum	Mean
1306	0.58	0.00	0.22	0.41	0.00	0.17
A13	1.35	0.00	0.77	1.17	0.00	0.64
A14	1.82	0.00	0.80	1.46	0.00	0.71
Average	1.25	0.00	0.60	1.01	0.00	0.51
	Total Acid					
1306	8.04	4.45	6.31	8.37	4.47	6.63
A13	6.64	3.45	5.51	7.98	3.45	5.77
A14	8.44	3.80	6.29	9.70	2.85	6.93
Average	7.71	3.90	6.04	8.68	3.59	6.44

TABLE XXVII

RATIO OF AVERAGE GASTRIC ACIDITY ON SKIMMILK TO THAT ON FORTIFIED SOYBEAN MILK

	Free Acid	Total Acid
Maximum	0.81	1.13
Minimum		0.92
Mean	0.85	1.07

In addition to being less in amount, free acid appeared in the samples at a later hour with the fortified soybean milk diet than with skimmilk, as shown in table XXVIII.

The points of maximum total acidity for the two rations appeared at the same time - approximately five hours after feeding, while the points of maximum free acidity occurred on

TABLE XXVIII

TIME OF APPEARANCE OF FREE ACID IN CALVES AFTER FEEDING SKIMMILK AND FORTIFIED SOYBEAN MILK

Calf	When fed skimmilk	When fed soybean milk
1306	3rd hour	4th hour
A13	2nd hour	4th hour
A14	2nd hour	3rd hour

the eighth hour with both rations.

These differences in maximum acidity cannot be attributed to differences in the nutrient contents (protein, carbohydrate, and fat), of the two diets since they are quite similar in this respect (table XXIX). Moreover, the fact that the same

TABLE XXIX

COMPARISON OF NUTRIENT CONTENTS (DRY WEIGHT) OF "FORTIFIED SOYBEAN MILK AND SKIMMILK

Ration	Protein	Carbohydrate	Fat	Nutritive ratio
Soybean milk	41.62	43.13	4.00	1.25
Skimmilk	36.25	51.50	2.00	1.54

ration excited (in other calves), a volume of pure gastric juice only slightly greater than did skimmilk, precludes any extensive secretagogic effect as a cause for the differences noted.

The hydrogen ion concentration of a mixture of soybean flour and water, as fed in the twelve-hour experiments, was found to be somewhat lower than that of skimmilk, (table XXX). Moreover, the addition of calcium chloride to the fortified soybean mixture made it more acid, and this effect was no doubt reflected in the total acidity of the stomach contents.

TABLE XXX

HYDROGEN ION CONCENTRATION OF SOYBEAN "MILKS" AND SKIMMILK

Ration	pH
Soybean flour in water	6.42
Fortified soybean milk	6.10
Skimmilk	6.70

CONCLUSIONS

Upon the basis of the results of this investigation to determine the gastric digestion of soybean flour, fed as a gruel to young dairy calves, the following conclusions seem warranted:

1. The Pavlov pouch, while a valuable instrument for studying the physiology of digestion in the ruminant stomach, is only of fair value in determining the rate of passage of food from the stomach and of the efficiency with which foods other than cow's milk can be utilized by the young calf.

2. The greater maximum half-hourly secretion and total volume of gastric juice produced in calves when fed a gruel of soybean flour in water, as compared to whole cow's milk, together with a more rapid decline in the half-hourly amounts of gastric juice, indicate that the soybean flour is somewhat more effective as a secretagogue in the stomach than whole cow's milk, and that the gruel passes from the stomach considerably more rapidly than does whole cow's milk.

3. A somewhat greater maximum half-hourly secretion of gastric juice, with a slightly more rapid decline, in calves fed a gruel of soybean flour mixed with skimmilk and water, and containing calcium chloride, as compared to skimmilk, showed that the soybean-cow's milk mixture as used in these experiments, passes from the stomach only slightly more rapidly than skimmilk.

4. Granting that the time required for evacuation from the stomach (abomasum), is a measure of the rate of gastric digestion, soybean flour is digested in the calf's stomach somewhat more rapidly than skimmilk.

SUMMARY

1. A group of four calves with Pavlov pouches was used in a series of twelve-hour experiments on whole cow's milk and simple soybean milk, with the volume of gastric juice being determined at half-hour intervals throughout the twelve hours. The secretion on soybean milk was 21.41 per cent greater during the first six hours of the twelve-hour period, but practically equal to the volume of secretion on whole cow's milk during the second six hours.

2. A group of four calves with Pavlov pouches, two of which had rumen fistulae in addition, was run for a continuous experiment of fourteen days - seven days on whole cow's milk, and seven days on simple soybean milk, the volume of gastric juice being determined at half-hour intervals throughout the fourteen days - day and night, and a full feed of whole cow's milk or simple soybean milk being given each eight hours. The results of this experiment showed that the maximum half-hourly volumes of gastric juice secreted on soybean milk were higher than the maximum volumes on cow's milk but that the total volume of secretion for the 24-hour period was less when the soybean milk was fed.

3. A group of five calves with Pavlov pouches was used in a series of sixteen-hour experiments in which fortified soybean milk was checked against skimmilk in determining gastric

digestion, by measuring the volume of gastric juice secreted on the two diets. The volume of secretion on fortified soybean milk was 7.71 per cent more than that on skimmilk during the first half of the sixteen-hour period and 3.28 per cent greater during the second half of the period.

4. A group of three calves with rumen fistulae was used to determine gastric acidity when fed fortified soybean milk and skimmilk. The fortified soybean milk was found to evoke a total acidity in the stomach, 6.62 per cent higher than skimmed cow's milk, with the point of maximum total acidity, 12.58 per cent higher than that for skimmed cow's milk. The free acidity of the soybean diet was lower by 15 per cent than that for skimmed milk, with the point of maximum free acidity, 19.2 per cent lower than that for skimmed milk. Free acid appeared the second hour after feeding, with skimmilk, and the fourth hour after feeding with fortified soybean milk. Samples taken up to sixteen hours after feeding contained soybean flour.

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